Understanding Chemical Sequential Extraction Method by Using Nuclear Magnetic Resonance and X–Ray Absorption Near Edge Spectroscopies for Phosphorus Fractionation of Lake Sediments

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By

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ABSTRACT

Phosphorus release from sediments contributes significantly to high phosphorus level in lake water and provides nutrient support to promote algal growth. To speed up the recovery of eutrophic lakes, it is necessary to limit phosphorus release from sediments. Accurate sedimentary phosphorus fractionation is a strong basis for understanding phosphorus release from sediments.

There are several techniques to study sedimentary phosphorus fractionation. Chemical sequential extraction (CSE) techniques are commonly used by industrial practitioners. However, it is doubtful that the P fractionation of the lake sediments studied using the Jensen and Thamdrup (1993) method is reliable. The reason is that the phosphorus fractions do not exactly correspond to the chemically defined compounds in the sequential phosphorus extraction. In order to further understand the Jensen and Thamdrup (1993) method, it is necessary to study P species in the supernatant and residue of each fraction. X-ray absorption near-edge structure (XANES) can provide direct information about the mineral phase of phosphorus in the sediments. Solution phosphorus nuclear magnetic resonance (^{31}P NMR) reveals direct molecular and structural characterization of organic phosphorus in the sediments.

This study enhanced the understanding of the Jensen and Thamdrup (1993) chemical sequential extraction method for studying the sedimentary phosphorus fractionation by using solution ^{31}P NMR spectroscopy and phosphorus K-edge XANES spectroscopy. The research using the chemical sequential extraction indicated that inorganic P was dominant in all sediments samples. Also, it suggested that calcium-bound P accounted for the largest proportion of the total P in every sediments sample. The solution ^{31}P NMR spectroscopy clearly identified orthophosphate, phytic acid, pyrophosphate, and polyphosphate in the sediments samples. The P K-edge XANES spectroscopy showed all of the sediments samples contained apatite and phytic acid. In addition, the study using the XANES identified apatite in the residue after the HCl extraction of Blackstrap #6; however it indicated no apatite in the supernatant of HCl fraction of both Blackstrap #3 and Pond #11.

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LIST OF ABBREVIATIONS

Al-P	Aluminum-Bound Phosphorus
BD	Bicarbonate Dithionite
Ca-P	Calcium-Bound Phosphorus
CSE	Chemical Sequential Extraction
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
EXAFS	Extended X-ray Absorption Fine Structure
Fe-P	Iron-Bound Phosphorus
FY	Fluorescence Yield
Labile-P	Loosely Sorbed Phosphorus
NMR	Nuclear Magnetic Resonance
NRP	Non-Reactive Phosphorus
Organic P	Organic Phosphorus
P	Phosphorus
P-NEXFS	Phosphorus Near Edge X-ray Fluorescence Spectroscopy
R.C.A.F	Royal Canadian Air Force
SRP	Soluble Reactive Phosphorus
SXRMB	Soft X-ray Microcharacterization Beamline
TEY	Total Electron Yield
TP	Total Phosphorus
XANES	X-ray Absorption Near-Edge Structure
XAS	X-ray Absorption Spectroscopy

CHAPTER 1 Introduction

1.1. Background

Phosphorus (P) is a vital biogenic element for aquatic ecosystem. Excessive phosphorus in lakes can accelerate freshwater primary productivity, leading to eutrophication. Eutrophication has negative impacts on ecosystem function. One of the negative environmental effects is the depletion of oxygen in the water, which may cause death to aquatic animals' lives (Wetzel 2001).

Phosphorus can be transferred from water to sediments through ion exchange, adsorption, and precipitation (Stumm and Morgan 1996); and also could be released from sediments (Furumai et al. 1989). Phosphorus release from sediments contributes significantly to high phosphorus levels in lake water and provides nutrient support to promote algal growth, which is referred to as internal phosphorus loading (Björk-Ramberg 1985). There are mobile phosphorus and immobile phosphorus in the sediments pools. Mobile phosphorus that can be easily released from sediments includes phosphorus in pore water, iron-bound phosphorus and some organic phosphorus compounds, while immobile phosphorus such as aluminum-bound phosphorus, calcium-bound phosphorus and refractory phosphorus are stable in sediments (Pettersson et al. 1988). Limiting phosphorus release is essential for speeding up recovery of eutrophic lakes. Phosphorus in sediments can be divided into a number of different fractions. Accurate sedimentary phosphorus fractionation is a strong basis for understanding phosphorus release from sediments.

There are several techniques to study sedimentary phosphorus fractionation. The chemical sequential phosphorus extraction technique has been commonly used for the determination of different phosphorus fractions of the sediments samples (Psenner et al. 1988; Jensen and Thamdrup 1993; Hupfer et al. 1995; Rydin and Welch 1998; Rydin 2000; Hansen et al. 2003; Lukkari et al. 2007). However, chemical sequential extraction involves sample destruction. A particular extractant tend to extract either part or some combination of P species (Shober et al. 2006). Thus, phosphorus fractions do not exactly correspond to chemically defined compounds in the sequential phosphorus extraction (Kar et al. 2011). Several researchers applied X-ray

absorption near-edge structure (XANES) to phosphorus speciation in soils, animal wastes, organic amendments (Hesterberg et al. 1999; Beauchemin et al. 2003; Sato et al. 2005; Ajiboye et al. 2008; Kar et al. 2011) and sediments (Brandes et al. 2007; Giguet-Covex et al. 2013; Li et al. 2014; Kraal et al. 2015). It has been well known that XANES has the advantage of being a more direct identification method and is nondestructive (no sample extraction is required). Although XANES can provide direct information about the mineral phase of phosphorus in soil, it has difficulty in identifying different types of organic phosphorus compounds (Peak et al. 2002). Studies of inorganic and organic phosphorus fractions in soils (Cade-Menun and Lavkulich 1997; Cade-Menun 2005; Turner et al. 2003; Turner 2008), manures (Cade-Menun et al. 2002) and sediments (Hupfer 2004; Reitzel et al. 2006) have been performed using solution phosphorus nuclear magnetic resonance (^{31}P NMR) with alkaline extracts. This technique provides direct molecular and structural characterization of organic phosphorus in alkaline solution, but does not present direct information about the mineral phase of phosphorus.

Although XANES and ^{31}P NMR are advanced techniques which provide direct molecular and structural characterization of P speciation, they are expensive and are not readily available for most of the industrial practitioners. It is costly for industrial practitioners to run XANES experiments, and it is not easy to get a beam time. Chemical sequential extraction (CSE) techniques are commonly used by industrial practitioners. However, it is doubtful that the P fractionation of the lake sediments studied using the Jensen and Thamdrup (1993) method is reliable. The reason is that the phosphorus fractions do not exactly correspond to the chemically defined compounds in the sequential phosphorus extraction. In order to further understand the Jensen and Thamdrup (1993) method, it is necessary to study P species in the supernatant and residue of each fraction.

After characterizing the P species extracted by the each extractant and the P species left in the residue of each fraction, it will help the researchers understand the mechanism of the extraction procedures of the CSE better. It will provide a complete information about P fractionation in the lake sediments by using the CSE for the industrial practitioners and researchers. It will provide another prospective for the improvement or modification of the Jensen and Thamdrup (1993) method.

1.2. Research Objective and Scope of Research

The objective of this study is to enhance the understanding of the Jensen and Thamdrup (1993) chemical sequential extraction (CSE) method for studying the sedimentary phosphorus fractionation by using solution ^{31}P NMR spectroscopy and phosphorus K-edge XANES spectroscopy.

This study does not only use the CSE, XANES and ^{31}P NMR to study P fractionation in the lake sediments, but also use ^{31}P NMR and XANES to characterize P speciation in the supernatant and residue of each fraction of the CSE.

This study is not assessing eutrophication states of the lakes nor controlling the P release from the lake sediments. This study does not discuss eutrophication remediation. This study does not intend to modify the Jensen and Thamdrup (1993) method.

1.3. Thesis Outline

Chapter 1 provides the background, the objective, and the scope of the research. Chapter 2 of this thesis presents a literature review of lake eutrophication and phosphorus in lake water and lake sediments. This chapter reviews chemical sequential extraction methods to characterize sedimentary phosphorus fractionation, and provides an introduction of ^{31}P nuclear magnetic resonance spectroscopy, and brief description of X-ray absorption near-edge structure spectroscopy. Chapter 3 presents materials and methodology. Chapter 4 presents and discusses the results of the experiments. The results principally include water contents and volatile solids of the sediments samples; total phosphorus in the lake sediments; the results of chemical sequential extraction, solution ^{31}P NMR spectroscopy, and P K-edge XANES spectroscopy. Chapter 5 presents conclusions derived from this study and recommendations for future work.

CHAPTER 2 Literature Review

2.1. Lake and Lake Sediments

Deep lakes are enclosed geographical bodies of fresh water, which are deep enough to thermally stratify into two or three layers during the summer in temperate regions. Shallow lakes are 3-4.5 meters deep or less, which the sunlight can reach the bottom. Natural lakes are generally found in mountainous areas, rift zones, and areas with ongoing glaciation. Artificial lakes are constructed for industrial or agricultural use, for hydro-electric power generation or domestic water supply, or for aesthetic or recreational purposes (Wetzel 2001).

The materials at the bottom of a lake are called lake sediments. Lake sediments may consist of a mixture of detrital sediment grains, algal or terrestrial organic matter and inorganically precipitated carbonate and saline minerals, along with numerous other fossil components (Schnurrenberger et al. 2003).

2.2. Eutrophication

Eutrophication is a process by which a body of water acquires a high concentration of nutrients, especially phosphorus and nitrogen. Eutrophication could be a natural stage as it gradually fills in with sediment eroded from its catchment and organic matter from its own metabolism. It starts from a low productivity stage then reaches a steady state of natural eutrophication as nutrients have been accumulating for thousands of years (Carpenter 1981).

Cultural eutrophication refers to the excessive plant growth resulting from nutrient enrichment by human activity. Human activities such as urbanization, transportation, irrigated farming, deforestation and forestation, land drainage, channelization and damming, and mining change the characteristics of lake (Peters and Meybeck 2000). Besides, human activities have accelerated the rate and extent of eutrophication through both point-source discharges and non-point loadings of

limiting nutrients, such as nitrogen and phosphorus, into aquatic ecosystems (Carpenter et al. 1998).

Excessive nutrient enrichment has many effects on freshwater ecosystem such as: increased biomass of freshwater phytoplankton and periphyton, shifts in phytoplankton species composition to taxa that may be toxic or inedible (e.g. bloom-forming cyanobacteria), changes in vascular plant production, biomass, and species composition. The excessive nutrient enrichment also makes the water body less aesthetically pleasing because it reduces water clarity, a bad taste, odor, and water supply filtration problems. Moreover, the phytoplankton consumes the dissolved oxygen in the water column, which may cause mortality of certain fish species and a shift towards less desirable fish species composition (Smith et al. 2006).

2.3. Phosphorus in Lake Water and Lake Sediments

2.3.1. Limiting Nutrients

One of the most effective long-term strategies for controlling cultural eutrophication of lakes is reducing the quantity of the nutrients entering the water body. Available evidence indicates that the phytoplankton biomass in a water body appears to be proportional to its nutrient load (Lee et al. 1978). The growth curve generally shows a linear increase with increasing nutrient concentrations up to a certain nutrient level. Beyond this point, no further increases in algal growth regardless of further nutrient increases (Jones et al. 1980). Assuming that algal growth is not controlled by a non-nutrient factor (light or temperature), reducing or 'limiting' the input of the nutrients should also limit the resulting algal biomass in the water body (Ryding and Rast 1989).

The limiting nutrient concept has its basis in the photosynthesis reaction. Stumm and Morgan (1996) presented the chemical needs of phytoplankton photosynthesis conceptually as follows:

$$CO_2 + NO_3^- + PO_4^{3-} \quad \text{(+ trace elements; sunlight)} \quad \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftharpoons}} \quad \text{algal protoplasm} + O_2$$

Redfield (1934) found that the average atomic ratio of three elements (carbon, nitrogen, phosphorus) in the plankton samples were 106C:16N:1P. In order to assess the relative

availability of nutrients, the concentrations of nutrients are compared with the molar Redfield ratio.

At a practical level, if one measures the nutrient concentration in lake water in similar units, the 16N: 1P atomic ratio reference value corresponds to a mass ratio of 7.2N: 1P. Thus, if the ratio of the measured concentrations is less than 7N: 1P, nitrogen is the potential limiting nutrient; if the ratio is greater than 7, phosphorus is the potential limiting nutrient. If the ratio is approximately 7, then either nutrients or some other factor may be limiting (Goldman 1979).

Nevertheless, different algal species have different optimum nutrient requirements (Grobbelaar and House 1995). An analysis of phytoplankton data in European lakes shows that cyanobacteria dominate lakes with relatively low concentration of soluble reactive phosphorus (SRP), while green algae dominate systems with higher SRP. Also, cyanobacteria dominance increases with total N concentrations (Schreurs 1992). In summary, cyanobacteria is more efficient in phosphorus uptake under phosphorus-limited conditions than green algae.

Practical use of the limiting nutrient concept assumes that a single nutrient required by algae will be the limiting factor at least during the period of water quality concern. Phosphorus is often chosen for primary attention in eutrophication control strategies, because it is relatively easily removed from municipal and other wastewaters by conventional water treatment methods, compared to other nutrients (Ryding and Rast 1989).

2.3.2. Different Phosphorus Forms in Lake Water

The total phosphorus of lake water consists of particulate phosphorus and dissolved phosphorus. Particulate phosphorus includes phosphorus in organisms, mineral phases of rock and soil, and phosphorus absorbed onto dead particulate organic matter. However, dissolved phosphorus consists of orthophosphate (PO_4^{3-}), polyphosphates, organic colloids and low molecular-weight phosphate esters. The most significant form of inorganic phosphorus is orthophosphate. A great proportion of phosphorus in fresh water is organic phosphates and cellular constituents (Wetzel 2001).

2.3.3. Different Phosphorus Fractions in Lake Sediments

The sediments can retain phosphorus by organic P reaching the sediments by sedimentation, inorganic P absorbed onto clays and aluminum and ferric hydroxides, and phosphate coprecipitates with iron, manganese, and calcium (Olila and Reddy 1993).

Phosphorus in sediments can be divided into a number of different fractions such as labile P, iron-bound P (Fe-P), aluminum-bound P (Al-P), organic-P, calcium-bound P (Ca-P), and residue P (Rydin and Welch 1998). Labile P is loosely sorbed P which is soluble reactive phosphorus (SRP) in pore water. Fe-P is a potentially mobile P, which is sensitive to low redox potentials. Al-P is relatively inert and is not redox sensitive. Organic-P includes bacteria-incorporated P. Ca-P is a stable fraction of sedimentary P. Residue P consists mainly of inert inorganic P fractions as well as refractory organic P. These fractions do not correspond exactly to chemically defined compounds but are characterized by elution medium and conditions of sequential P extraction. Organic-P is often liberated by strong acid or alkaline solutions in sedimentary P extraction (Pettersson et al. 1988).

2.3.4. External Phosphorus Sources for Lake Water

There are two common external P sources of nutrients and organic matter: point and nonpoint sources. Point sources are wastewater effluent (municipal and industrial), runoff and leachate from waste disposal systems, runoff and infiltration from animal feedlots, runoff from mines, oil fields, unsewered industrial sites, overflows of combined storm and sanitary sewers, runoff from construction sites less than 20,000 m^2 (220,000 ft^2) and untreated sewage (Carpenter et al. 1998).

Nonpoint sources are runoff from agriculture/irrigation, runoff from pasture and range, urban runoff from unsewered areas, septic tank leachate, runoff from construction sites >20,000 m^2, runoff from abandoned mines, atmospheric deposition over a water surface and other land activities generating contaminants (Carpenter et al. 1998).

2.3.5. Phosphorus Release from Lake Sediments

Depending on hydrodynamic and biotic mechanisms, dissolved phosphorus can transport from the sediments to the lake water. In deep lakes, because of the steep concentration gradients of

phosphorus, molecular diffusion is a primary transport into the overlying anaerobic water. In shallow lakes, currents from wind-induced water turbulence can disrupt gradients and resuspend sediment particles. Moreover, disturbance of sediments by bottom feeding fishes can cause appreciable bioturbation of sediments. In addition, the metabolism and growth of plants living on and within the sediments can either suppress or enhance the transport of phosphorus across the sediments-water surface (Wetzel 2001).

The dynamics of nutrients is different between shallow lakes and deep lakes. In deep lakes, thermal stratification occurs during summer and winter. Nutrients present in the upper water layer will be transported to the bottom water. In spring and autumn, the nutrients from sediments in the bottom water are available to the upper water layer. Because there is no stratification in shallow lakes, the exchange of material between sediment and water is continuous (Jeppesen et al. 1997).

The shallow lakes produce lots of organic matters during the summer. Decay of this organic matter causes the lower layers to stay in an oxygen depletion condition. Mortimer (1942) found that the degree of oxidation-reduction at the sediment surface determines the movement of phosphorus between sediments and lake water. If the sediment surface is oxidized, iron is present in ferric state precipitated in a colloidal structure, which prevents phosphate exchange. If the sediments surface becomes anaerobic, ferric is changed to ferrous, the colloidal structure breaks down and phosphate moves from sediments into the overlying water. Also, Jarvis (1980) pointed out that decomposition of organic matter in surficial sediments releases phosphate into overlying water column. In addition, the sediments disturbed by agitation from turbulence can increase the rate of phosphorus release from lake sediments (Zicker et al 1956). Resuspension of inorganic sediments particles is mainly caused by wave action. These resuspended particles can be seen as an internal source of phosphate (Ogilvie and Mitchell 1998).

2.3.6. Internal Loading of Phosphorus and Needs of Determining Phosphorus Fractionation in Sediments

The phosphorus content in lake sediments can be several orders of magnitude greater than that in the overlying water (Boström and Pettersson 1982). Algae are able to utilize phosphorus form in the sediments for growth. It has been shown that without sediments phosphorus sources, the phosphorus content of the water limits algal growth under experimental conditions (Björk-

Ramberg 1985). These results stress the importance to study P fractionation in lake sediments (internal loading) to understand the ability of the sediment to retain phosphorus and the effect of phosphorus transport back to the water.

2.4. Chemical Sequential Extraction

2.4.1. Chemical Sequential Extraction Methods

Chemical sequential extraction is widely used to determine different fractions of phosphorus in sediments (Pettersson et al. 1988). A best example of early extraction schemes is presented by Chang and Jackson in 1957 who partitioned sediment P into labile P, aluminum-bound P, iron-bound P, calcium-bound P, reductant soluble P, occluded P, and organic P. Williams and coworkers (1967) pointed out the shortcomings of the Chang and Jackson procedure, which included the extraction of iron-bound P besides aluminum-bound with NH_4F and the resorption of phosphate due to the formation of CaF_2. The method of Chang and Jackson (1957) was further enhanced by other researchers.

Hieltjes and Lijklema (1980) separated sediment P into labile P (NH_4Cl-P), iron- and aluminum-bound P (NaOH-rP), calcium-bound P (HCl-P), and a residue P fraction. However, iron-bound P and aluminum-bound P were not separated in this sequential P extraction scheme, and organic P was not identified but included within NaOH-rP and/or the residue P fraction (Hieltjes and Lijklema 1980). Pettersson et al. (1988) suggested that the weaknesses of Hieltjes and Lijklema method are the dissolution of small amounts of iron-bound P and aluminum-bound P by NH_4Cl.

In 1988, Psenner et al. presented a phosphorus fractionation scheme designed to separate water-soluble P (H_2O-P), P bound to iron and manganese hydroxide or reluctant soluble P (BD-P), iron- and aluminum-bound P (NaOH-rP), calcium-bound P (HCl-P), and refractory P ($NaOH_{85}$-P). However, Psenner's procedure has the drawback of resorption of phosphate by carbonates in calcareous sediments (Pettersson et al. 1988).

In 1993, Jensen and Thamdrup modified the sequential extraction scheme proposed by Psenner et al. (1988). In the first step, the extraction with H_2O was replaced by 0.46 M N_2-purged NaCl solution. In the third step, 0.1 M NaOH was chosen to extract aluminum-bound P and Organic P. Also, it added ignition and boiling with adding 1 M HCl after fourth step to extract the Residue P.

In addition, Jensen and Thamdrup (1993) concluded the BD-reagent was very specific for iron-bound P.

In order to characterize various P-species in sediments, Hupfer et al. (1995) modified the sequential extraction scheme of Psenner et al. (1988). All extractions were conducted at room temperature after modification. The extraction with H_2O was replaced by an extraction with 1 M NH_4Cl. The last extraction with hot NaOH was omitted. Instead, the P content of solid material remaining after extraction with HCl was determined after persulfate ($K_2S_2O_8$) digestion. Hupfer et al. (1995) also found that changes in BD-P were highly significantly correlated with changes in reductant soluble iron (BD-Fe).

Rydin and Welch (1998) and Rydin (2000) improved Hieltjesand Lijklema's (1980) method with two modifications: an anoxic bicarbonate dithionite (BD) step and a digestion step added to the NaOH extraction. Thus, Rydin and Welch (1998) and Rydin (2000) separated sediment P into labile P (NH_4Cl-P), iron-bound P (BD-P), aluminum-bound P (NaOH-rP), organic P (NaOH-nrP), calcium-bound P (HCl-P), and a residue P fraction. These inorganic P forms (BD-P and HCl-P), although operationally defined, were successfully separated in the P fractionation procedure (Rydin 2000). However, one cannot rule out the possibility that some of the P detected as NaOH-rP was actually organic P hydrolyzed with the relatively strong extractant used (0.1 M NaOH). This is one disadvantage of using relatively strong extractants in the fractionation procedure (Golterman 1982). NaOH-nrP was the only fraction that could not be separated into mobile or permanently bound forms. In the sediment profile more than 50% of the NaOH-nrP was lost with depth corresponding well to the results from the experiments (Rydin 2000).

Hansen et al. (2003) advanced the P fractionation scheme presented by Psenner et al. (1988) with two modifications: 1.5 mL 2 M H_2SO_4 added to the NaOH extraction step to determine humic acid-bound P and a sediment combustion step to determine the residue P fraction. Psenner et al. (1988) suggested that Al-bound P appeared in the NaOH fraction. Hansen et al. (2003) also indicated recovery of added aluminum along with an increased P pool in the NaOH fraction. The low P concentration in the BD fraction can be explained by the fact that aluminum hydroxides are capable of adsorbing iron-bound P, which is mobilized when ferric is reduced (Cooke et al. 2005).

Lukkari et al. (2007) modified the last step of Jensen and Thamdrup's (1993) procedure to determine residual or recalcitrant organic P: instead of the combusted (2 h at 520°C) sediment residues being boiled in 0.5 M HCl (10 min), they were combusted for 2 h at 550°C in ceramic vials, cooled, weighed, and removed quantitatively back to the extraction tubes. The vials were carefully rinsed with portions of the extraction solution to be used in the last step, the rest of the 1 M HCl extractant was added to the tubes, and the sediment residues were extracted for 16 h.

After reviewing the modifications of the sequential phosphorus fractionation by Chang and Jackson (1957), Hieltjes and Lijklema (1980), Psenner et al. (1988), Jensen and Thamdrup (1993), Hupfer et al. (1995), Rydin and Welch (1998), Rydin (2000), Hansen et al. (2003), and Lukkari et al. (2007), the P extraction scheme proposed by Jensen and Thamdrup (1993) was used in this study.

2.4.2. Sequential Phosphorus Extraction Scheme Proposed by Jensen and Thamdrup

The procedure of Jensen and Thamdrup (1993) was chosen because it provides more detailed information on mobile and immobile organic P and redox-sensitive P. This information plays an important role in risk assessment of internal P loading. The sediments intended to be studied are known to be poor in calcium carbonate, but rich in humic matter and consequently in organic P. Moreover, reagents used in Jensen and Thamdrup (1993) are more dilute, which disturbs the material less and thus gives more reliable estimates for the bioavailability of sediment P.

To explain in details, the advantages of the chemical sequential method proposed by Jensen and Thamdrup (1993) are the following: First, the scheme uses 0.46 M NaCl instead of 1 M NH_4Cl for extraction of the loosely bound phosphorus in step 1. 0.46 M NaCl was used because it was found to extract less than twice the amount of dissolution of calcium-bound P than 1 M NH_4Cl (Pettersson et al. 1988). Also, the use of a NaCl wash after each extraction step overcomes the reabsorption of phosphate (Jensen and Thamdrup 1993). Moreover, the molarity of 0.1 instead of 1.0 was chosen for NaOH in the method of Jensen and Thamdrup 1993. The more dilute NaOH is expected to more sensitively extract the easily hydrolysable organic P compounds than the 1.0 M NaOH will (Hupfer et al. 1995). In addition, this extraction scheme was found to be very reproducible and was applied for studying seasonal variations in sedimentary P fractionation.

The disadvantage of this method is that BD-srP was slightly overestimated for freezing sediment samples. This happened because some non-reactive phosphorus (NRP) that was supposed to be extracted by NaOH was hydrolyzed in the freezing process and it showed up as BD-srP in the extraction scheme instead (Lukkari et al. 2007).

2.5. Phosphorus Nuclear Magnet Resonance (^{31}P NMR) Spectroscopy

2.5.1. Basics of NMR Spectroscopy

The basics of nuclear magnet resonance (NMR) spectroscopy can be found in various textbooks (Nelson 2003). Atomic nucleis, such as ^{1}H, ^{13}C, ^{14}N, ^{19}F, ^{31}P, etc, possess an intrinsic angular momentum or spin. When these nuclei are placed in a strong magnetic field of an NMR spectrometer, the nuclei will align with the magnetic field (like tiny bar magnets) so that the nuclei are pointing either "toward" or "against" the direction of the magnetic field, corresponding to two energy levels of the nuclei. Then the nuclei are irradiated with such electromagnetic frequency that the photons (quanta) have energy exactly equal to the separation of the two levels of energy. If the nucleus is in the lower level of energy state it can be promoted to the upper level. If it is in the higher level of energy state it can be stimulated by the irradiation to emit a photon and fall to the lower state. Quantum mechanics indicates that if a particle is moving with periodic motion (oscillating), then the particle can absorb electromagnetic radiation if the frequency of that motion exactly matches the frequency of the radiation. This is nuclear magnetic resonance that can be recorded by the NMR spectrometer as a peak in the spectrum.

2.5.2. Solid-State ^{31}P NMR Spectroscopy

Solid-state ^{31}P NMR study of environmental samples uses 300 or 400 MHz spectrometers, spin rates of 5-10 kHz, and spectral widths as wide as 400 ppm (200 ppm to -200ppm). Experiments may collect as many as 170000 scans, and may last as long as 48 h. An external standard of 85% H_3PO_4 is used, and hydrolysis caused by high temperatures is not a concern (Cade-Menun 2005).

Solid phase NMR is more limited than solution NMR for studying P pools due to poor spectral resolution. The poor spectral resolution of solid state NMR results from the presence of paramagnetic ions in the sample (Cade-Menun 2005). A comparison of solid-state and solution ^{31}P NMR spectra for the same samples is shown in Figure 2.1.

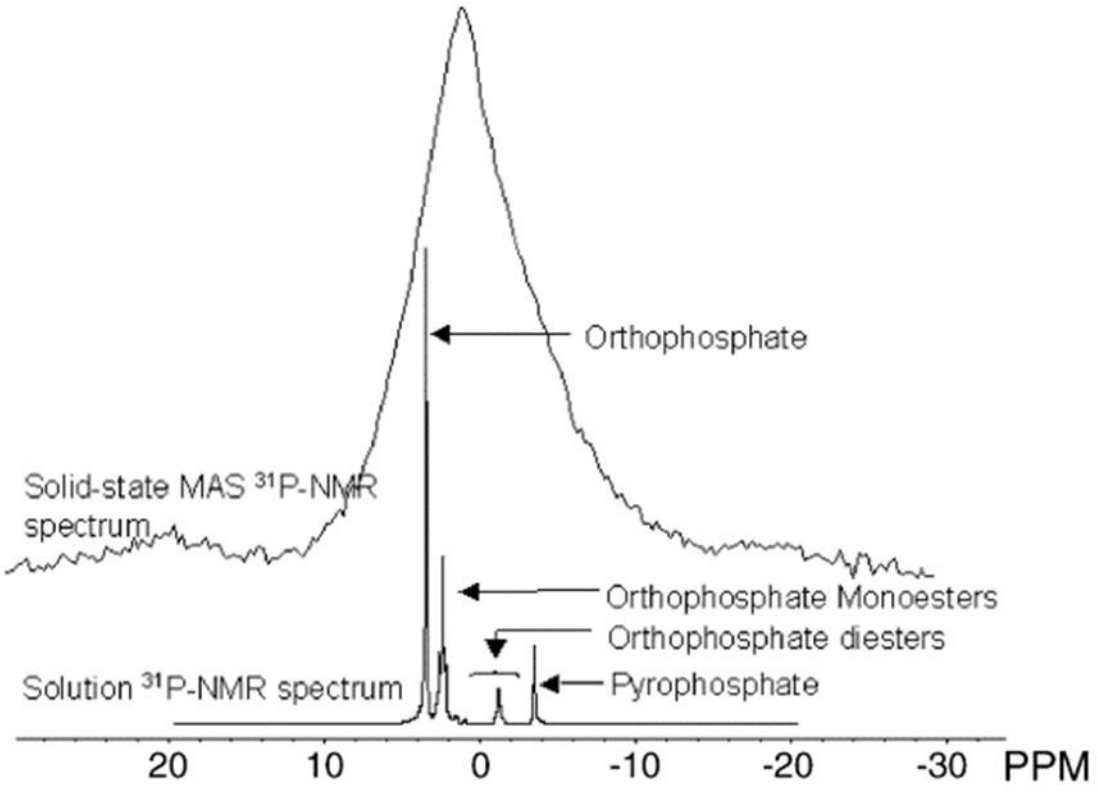

Figure 2.1. A comparison of solid-state and solution ^{31}P NMR spectra for the same sample (Cade-Menun 2005)

The peaks of the solid-state spectrum are broad, each of which overlaps the chemical shifts of several P nuclei. In contrast, peaks in the solution spectra are narrow, allowing P nuclei to be more easily identified.

2.5.3. Solution ^{31}P NMR Spectroscopy

The phosphorus-31 NMR spectroscopy has substantially advanced the knowledge of phosphorus (P) compound content in soil, water and other environmental samples. The solution ^{31}P NMR is one of the most routine NMR techniques, because ^{31}P has an isotopic abundance of 100% and a relatively high gyromagnetic ratio. The ^{31}P nucleus also has a spin of $\frac{1}{2}$, making spectra relatively easy to interpret. Most solution ^{31}P NMR experiments have used a 500 MHz magnet with a 10mm broadband probe for environmental studies (Cade-Menun 2005).

For solution ^{31}P NMR, processing software converts the data to a frequency-domain form with signal intensity recorded as a function of frequency. Frequency is expressed as chemical shift (δ),

which is the measure of the position of a resonance signal relative to the standard (85% phosphoric acid). Chemical shift is independent of the laboratory magnetic field. And it is defined by Equation 2.1:

$$\delta = \frac{(Vs-Vr)*10^6}{Vr} \quad [2.1]$$

where [Vs] and [Vr] are the frequencies of the sample and reference standard (85% phosphoric acid), relative to that of the applied magnetic field (Wilson 1987).

The chemical shift of the standard is set at 0 ppm (Hoffman 2003). In the environmental science application of solution ^{31}P NMR, a library of chemical shifts of model P compounds, which is shown in Table 2.1, is used to identify the compounds in the experimental spectrum (Cade-Menun 2005).

Solution ^{31}P NMR spectroscopy has been used successfully to study the P species of the soil (Cade-Menun and Preston 1996; Cade-Menun et al. 2002; Turner et al. 2003; Turner 2008) and the lake sediments (Hupfer 2004; Reitzel et al. 2006). This technique provides direct molecular and structural characterization of organic P in alkaline solution. Cade-Menun and Preston (1996) reported that the use of a chelator ethylenediaminetetraacetic acid (EDTA), together with alkaline extractants like NaOH improves the recovery of total P from the sample. Turner et al. (2003) reported solution ^{31}P NMR chemical shifts of model P compounds, including inorganic phosphates, orthophosphate monoesters and diesters, phosphonates, and organic polyphosphates, determined in a standardized soil P extractant (0.25 M NaOH and 0.05 M EDTA). Hupfer et al. (2004) found that polyphosphate (Poly-P) was detected in sediments from a large variety of lakes with different trophic state with the use of ^{31}P NMR spectroscopy. The transformation of organic P compounds and Poly-P can contribute significantly to the release of P during diagenesis.

Reitzel et al. (2006) expanded the understanding of organic P forms in three lake surface sediments by using sequential P extraction and ^{31}P NMR spectroscopy. The results indicated that non-reactive P from the dystrophic lake was dominated by potentially recalcitrant P groups such as orthophosphate monoesters. Whereas the non-reactive P in the two more productive lakes contained polyphosphates, pyrophosphate, and organic P groups such as P lipids and DNA-P that may be important in re-mineralization and recycling to the water column.

Table 2.1. Chemical shift references for alkaline extracts (pH 12) of biological P compounds (Cade-Menun 2005)

Compound	Chemical shift (ppm)
Phosphonates	20
Aminoethyl phosphonates	20
Phosphonolipids	18
Aromatic phosphonic acid esters	12 to 14
Aromatic diesters	7.4
Orthophosphate	5.7 to 6.1
Orthophosphate monoesters	6-3
Myo-inositol hexakiphosphate (phytic acid)	5.85,4.92,4.55,4.43
Glucose-6-phosphate	5.4
Mononucleotides	4.78 to 4.32
B-glycerophophate	4.85
Ethanolamine phophate	4.71
Scyllo-inositol hexakisphosphate	4.14
Choline phosphate	4.05
Glucose-1-Phosphate	3.2
Orthophosphate diesters	2.5 to -1.0
Teichoic acids	2.5 to 1.2
Phosphatidyl ethanolamine	1.75
Phosphatidyl serine	1.57
Phosphatidyl choline	0.78
RNA	0.54
DNA	0
DNA	-0.37
Polyphosphate terminal P group	-4
Pyrophosphate	-4 to-5
ATP or ADP α-phosphate	-10
Polyphosphates	-19 to-21
ATP β-Phosphate	-19.68

There are several limits in using ^{31}P NMR to determine the P pools. First, solution NMR does not present molecular information about the mineral phase of P, which is identified only as orthophosphate. Second, using the alkaline extract to hydrolyze some labile organic P into orthophosphate may lead to the underestimation of organic P pools in the sample (Turner 2004). Third, the recovery of polyvalent cations in the extract used for NMR analysis does not provide a direct evidence of their association with either the organic or the inorganic P pools.

2.6. X-ray Absorption Near-Edge Structure Spectroscopy

2.6.1. Synchrotron Facility and X-ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) is one of the synchrotron radiation-based techniques. A schematic of a synchrotron radiation facility is shown in Figure 2.2. A synchrotron light source begins with an electron gun, which results in electrons being lifting off and being propelled down a linear accelerator. Then electrons enter a circular-shaped booster ring, where they are accelerated at approximately the light speed. Finally, they enter the storage ring, where they circulate for nearly 20 hours. There are special bend magnets around the ring, which help electrons keep to their circular path and keep them bunched together and focused. Synchrotron light is produced when the electrons change direction around the ring. In synchrotrons, when the bending magnets alter the accelerated electrons, a fan of radiation is given off (known as synchrotron light). This radiation branches off the storage ring, and enters laboratories or beamlines (Singh and Gräfe 2010).

X-ray absorption spectroscopy describes the interaction between X-rays, atoms and molecules. When an atom absorbs X-ray photons, a core level (K, L or M) electron is ejected into the continuum, leaves an empty electronic level (core hole) behind (Figure 2.3), and the atom becomes ionized and excited state. In the process of deexcitation (Figure 2.4) followed after the excitation, the empty core hole is filled by a higher level electron, and subsequently an X-ray fluorescence or an auger electron is emitted. The energies of the photon absorbed or emitted photoelectron or the intensity of the emitted fluorescence is measured in a high vacuum absorption chamber. The XAS spectrum is created by the recorded signal over a range of incident photon energies (Ajiboye et al. 2007).

A typical XAS spectrum is made of the near edge-region: X–ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) .The XANES region extends over a range of about 100 eV (± 50 eV of the edge), between the edge region and the EXAFS region (Figure 2.5). The XANES spectra reports electronic structure and symmetry of the metal site, and the EXAFS reports numbers, types, and distances to ligands and neighboring atoms from the absorbing element (Yano and Yachandra 2009).

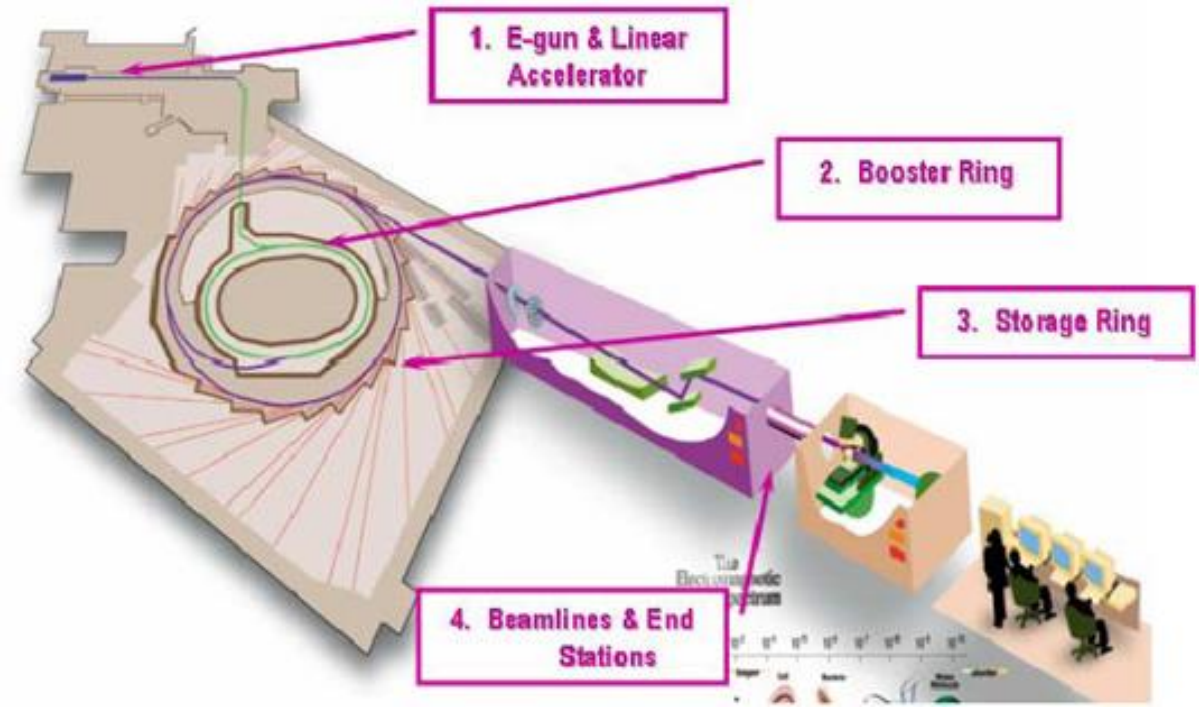

Figure 2.2. Synchrotron radiation facility schematic (Adapted from http://dmttest.usask.ca/education/whatis.php)

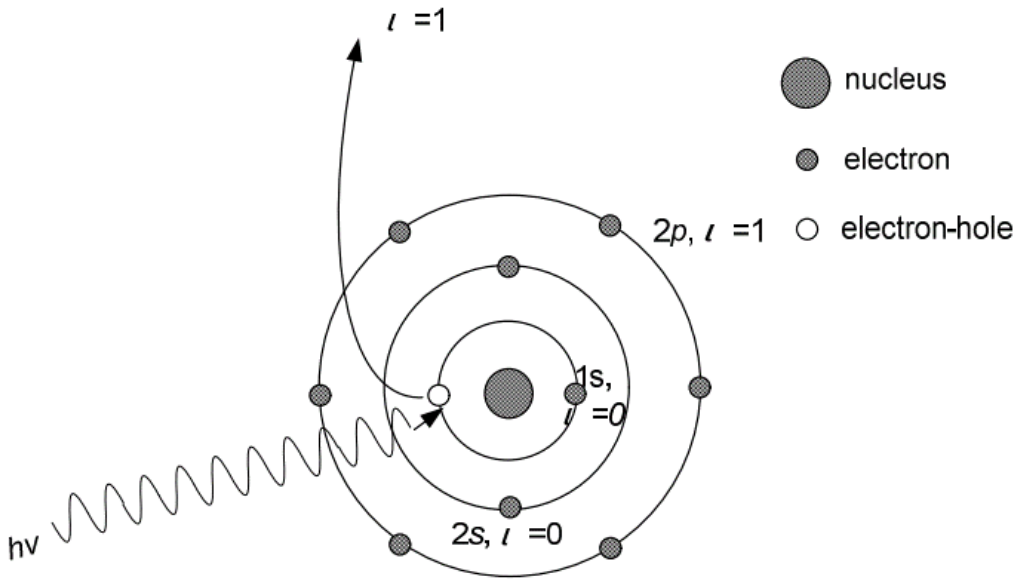

Figure 2.3. Core level position of an isolated atom (Sutton 1993)

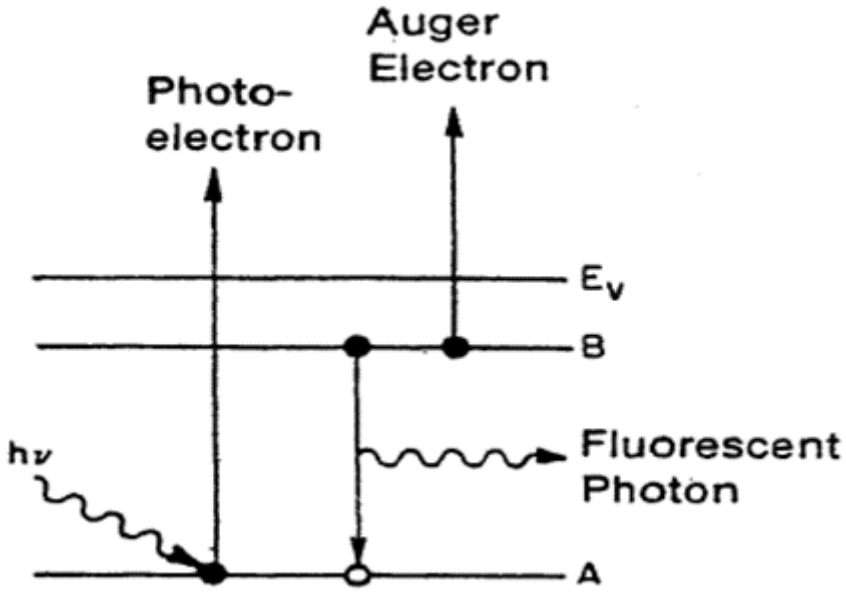

Figure 2.4. Electronic transition from atomic core level 1s to unoccupied molecular states

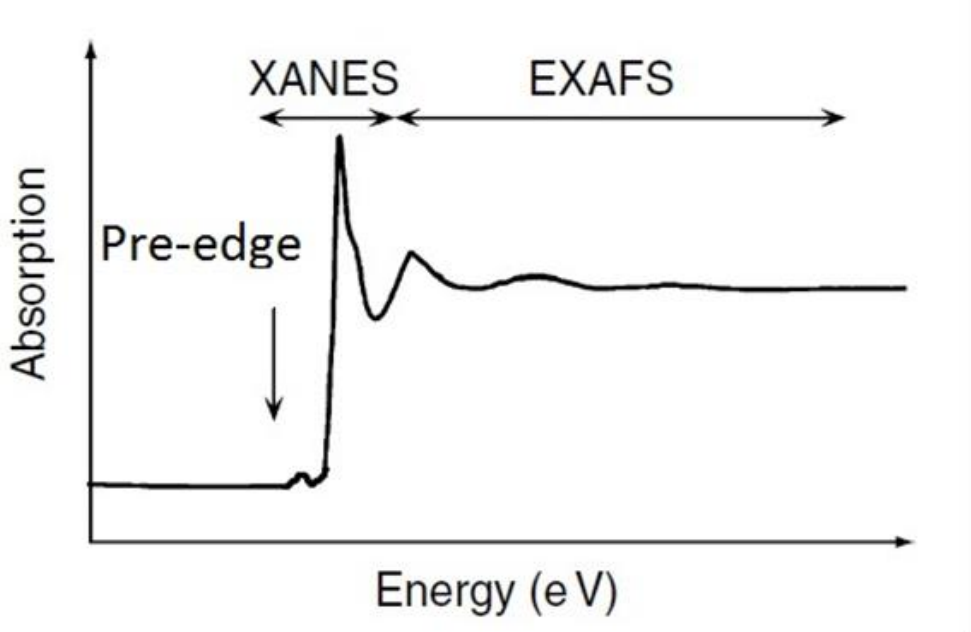

Figure 2.5. X-ray absorption spectroscopy spectrum

2.6.2. Detection Modes of X-ray Absorption Spectroscopy

There are three detection modes commonly used in XAS: transmission, fluorescence yield (FY), and total electron yield (TEY). In the first detection mode, X-rays are attenuated as they are transmitted through the sample. The intensity ratio of the incoming X-ray and the outgoing X-ray is proportional to the exponential of the absorption coefficient times the thickness. The spectrum will show a sudden decrease in transmittance as the beam of scanning X-ray meets the absorption edge. The obtained transmittance spectra are usually converted to absorption spectra afterward. X-ray absorption is described by Equation 2.2.

$$A = \ln(\frac{I_0}{I_f}) \qquad [2.2]$$

where I_0 is the incident photon flux and I_f is the transmitted photon flux.

The disadvantage of the transmission mode is that it is limited to moderately concentrated samples (eg. greater than 500 ppm). In cases of certain samples or solvents, the incoming X-ray photons could be nearly completely absorbed.

The second detection mode, X-ray fluorescence, is best suitable for less concentrated and thin samples. The intensity of X-ray fluorescence is described by the relationship:

$$\mu(E) \propto (\frac{I_f}{I_0})$$

The intensity of X-ray fluorescence signal (I_f) is directly proportional to the X-ray absorption cross-section of the sample. In practice as a beam of X-ray is shined at high concentration of the element of interest, self-absorption may be observed in the fluorescence spectrum (Ajiboye et al. 2007).

Another detection method used in XAS is total electron yield (TEY) especially for light elements with low fluorescence yield. The TEY includes the initial photoelectron created by the excitation process and any Auger electron created by decay processes of the core-hole excited state. Similar to X-ray fluorescence detection, the intensity of the TEY (I_e) is proportional to μ (E) of the sample.

As the total electron or fluorescence photon yield emitted from the sample is proportional to the absorption coefficient, both techniques are equivalent to a transmission measurement (Kasrai et al. 1993). In the soft X-ray region, atoms relax after X-ray absorption primarily by emission of an Auger electron. One can measure a signal related to the absorption cross section by recording the Auger electron yield. Because the electron escape depth is short (<100 A), TEY is very surface sensitive. The X-ray fluorescence detection is the dominant technique for hard X-ray experiments.

2.6.3. X-ray Absorption Near-Edge Structure (XANES) Spectroscopy

The spectrum usually generates an 'absorption edge' at the photon energy that approaches the binding energy of the orbital (K, L or M) electrons. The names of the absorption edges are given according to the principal quantum number, n, of the excited electrons. Energies of absorption edges reveal the identity of the corresponding absorbing elements.

Different core electrons have distinct binding energies; consequently, if one plots the X-ray absorbance of a specific element as a function of energy (Penner-Hahn 2005), the resulting spectrum will appear similar to Figure 2.6, which K, L, and M edge transitions are identified, and the L edge fine structures are shown.

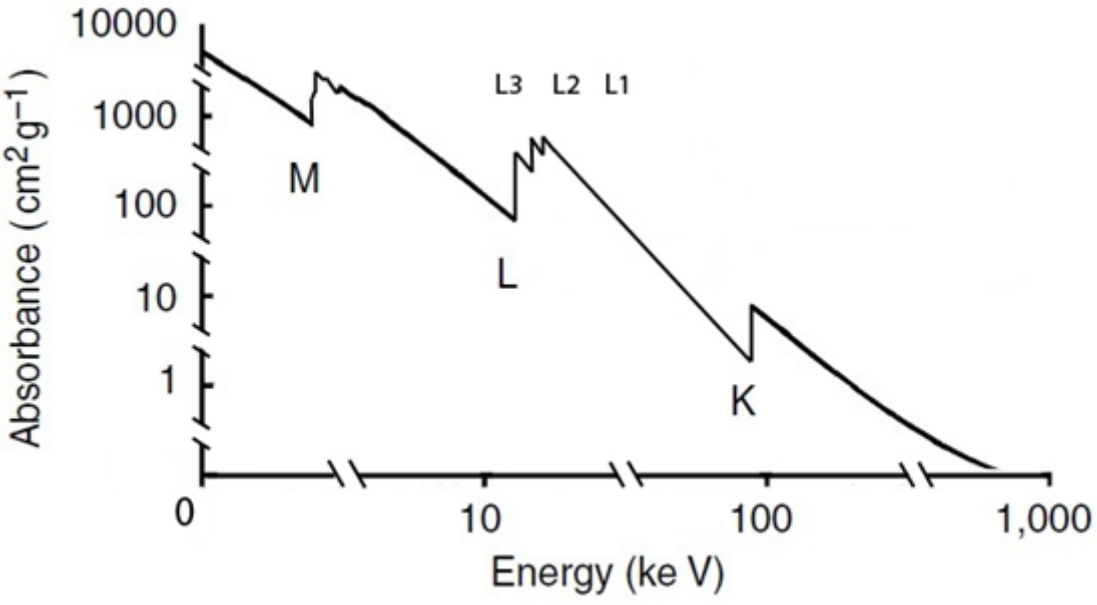

Figure 2.6. X-ray absorption spectrum of a transition metal atom (Penner-Hahn 2005)

2.6.4. XANES Applications in Characterizing Phosphorus Speciation

Application of the XANES has been widely used in studying P compounds in environmental samples. Not only it is an investigation tool that is nondestructive, oxidation states can be determined, but also it provides the local chemical and structural environment of the element (Fendorf et al. 1996).

Ingall et al. (2010) conducted P K-edge XANES spectroscopy on phosphate mineral standards. The study indicates minerals are notable using P XANES spectra with a combination of pre-edge position, peak shapes and post-edge features. Shared spectral features are observed in minerals with compositions dominated by the same specific cation. The principal K-edge peak energies in the XANES spectra of all phosphorus minerals in the study are between 2152.9 eV and 2154.1 eV (Brandes et al. 2007). The peak energies of various phosphate mineral are slight different: apatite minerals (2153.0 ± 0.1 eV), aluminum phosphate minerals (2153.6 ± 0.2 eV) and oxidized iron and manganese phosphates (2153.9 ± 0.2 eV).

For apatite-group minerals, a distinctive shoulder or widening on the main absorption edge peak centered at approximately 2155.6 eV, and higher-energy secondary peaks at 2163.3 and 2170 eV are the identifying features. Non-apatite calcium phosphates display similar features with apatite-group minerals; however, the shoulders for latter are not nearly as distinct as those detected in apatite-group minerals (Ingall et al. 2010).

Aluminum phosphate minerals have a narrow K-edge peak (~ 0.9 eV full width at half-maximum), but lack the shoulder seen in the calcium phosphate mineral spectra. A distinguishing feature to aluminum phosphate mineral spectra is the secondary peak centered at approximately 2175 eV. For the oxidized iron and manganese phosphate minerals, a unique feature is the pre-edge peak present at 2150.1 eV (Ingall et al. 2010).

To date, a number of papers have used P K-edge XANES spectroscopy to characterize P speciation directly in soil samples (Hesterberg et al. 1999; Beauchemin et al. 2003; Sato et al. 2005; Ajiboye et al. 2008; Kar et al. 2011). Kar et al. (2011) used a combination of sequential chemical extraction and synchrotron XANES spectroscopy on the solid-phase residues after each extraction step to compare the speciation of P in soils amended with either biosolids or an

inorganic fertilizer. It indicates that although sequential extraction steps may remove the same broad class of P from soil, the solubility and precise chemical speciation of that P may be quite different.

A few other publications have focused on XANES speciation of P in animal wastes and organic amendments (Peak et al. 2002; Shober et al. 2006; Ajiboye et al. 2007). Shober et al. (2006) identified the P species in dairy manures, poultry litters, and biosolids by using P K-edge XANES and chemical fractionation techniques. His results indicated that the specific chemical extractant (or combinations of extractants) often overestimates or underestimates the amount of P species determined from XANES fitting. Although chemical fractionation techniques display different forms of P in these materials, the differences between each form of P should be interpreted based on P speciation data obtained using more advanced analytical tools (Shober et al. 2006).

Ajiboye et al. (2007) used the combination of the three techniques: sequential chemical extraction, solution ^{31}P NMR spectroscopy, and P K-edge XANES spectroscopy to study the molecular characteristics of P in organic amendments (anaerobically digested biosolids, hog, beef, and poultry manures). They found the combination of the three techniques provided molecular characterization of P in organic amendments that would not have been possible with just one or a combination of any two of these techniques.

However, just a few publications have focused on speciation of phosphorus in sediments (Brandes et al. 2007; Giguet-Covex et al. 2013; Li et al. 2014; Kraal et al. 2015). Brandes et al. (2007) collected phosphorus near edge X-ray fluorescence spectroscopy (P-NEXFS) data on phosphorus containing phases including organic and inorganic compounds and minerals. Significant polyphosphate-dominated regions were presented in a marine sediment sample, which supported the idea that such phases can play an important role in marine P cycling (Brandes et al. 2007). In addition, it indicated that the combination of fluorescence mapping and P-NEXFS data collection on fine particles provides a powerful new tool for environmental phosphorus studies.

Giguet-Covex et al. (2013) used XANES spectroscopy as a tool to trace phosphorus transformation during soil genesis and mountain ecosystem development from lake sediments. The study suggested phosphorus geochemistry during the main step of soil genesis was clearly

recorded in lake sediments. Early leptosols were dominated by apatite. Low weathered cambisols contained P mainly adsorbed on iron oxides. Highly weathered podzols consisted of large amounts of P on Al/Fe/clay organic complexes. It also indicated P K-edge XANES spectroscopy is particularly relevant as qualitative method to study P species in soils and lake sediments at high spatial resolution (Giguet-Covex et al. 2013).

Li et al. (2014) characterized phosphorus speciation of Chesapeake Bay sediments using chemical extraction, ^{31}P NMR, and EXAFS spectroscopy. Sequential P extraction displayed that P in the sediments was composed primarily of ferric Fe-bound P and authigenic P, which was further confirmed by solid-state ^{31}P NMR, XANES, and EXAFS analyses. Also, it suggested that the sediments from the middle site contained high amounts of organic P such as monoesters and diesters by solution ^{31}P NMR results. Besides, it was hard to exclude the presence of iron phosphate from P K-edge XANES; however, Fe EXAFS enabled to identify the changes in Fe mineral composition in response to imposed redox condition in the middle site sediments (Li et al. 2014). Furthermore, Li et al. (2014) found that although the chemical sequential extraction technique is a useful tool and allows for comparisons of P pools among different sites and depth, some of the chemical fractions could represent more P pools than those P species chemically defined. Spectroscopic techniques are a good addition to sequential extraction techniques, because they provide direct identification of important P phases (Li et al. 2014).

Kraal et al. (2015) demonstrated the possibilities of P K-edge XANES as a bulk P speciation tool for marine sediments. The P K-edge XANES results confirmed the reliability of the sequential chemical extraction procedure to determine the iron (Fe-) associated P and calcium phosphate minerals. Moreover, the use of the combination of sequential chemical extractions and P K-edge XANES spectroscopy can provide further insight into the nature of the operationally defined P phases. However, Kraal et al (2015) found distinguishing between various organic P phases is challenging. It is suggested that complementary techniques such as the ^{31}P NMR would be required for a detailed study of the organic P component.

2.6.5. XANES Limits in Phosphorus Determination

XANES has limitations in characterization of P speciation as follows:

- The fluorescence signals of samples are very weak because of the low concentration of P in most soils and sediments. Thus, it needs long data collection times to obtain high quality XANES spectra (Kelly et al. 2008).
- For low P concentrations, TEY mode detection is less sensitive than fluorescence-mode detection (Ajiboye et al. 2008).
- Limited specificity: P is heterogeneously distributed within a sample, focusing the beam on P-enriched spots improves the signal to noise ratio (Lombi et al. 2006).
- Limited sensitivity means that the spectra are lacking strong unique spectral features. It is not sensitive enough to distinguish multiple species of organic phosphorus because of no unique feature in organic P spectra (Singh and Gräfe 2010). Also, there is uncertainty in selecting standards for linear combination fitting analysis. Fitting models may not reflect the actual soil speciation (Beauchemin et al. 2003).
- Mixing is likely to be more problematic at the P-K edge, where the low-energy synchrotron X-ray beam will not fully penetrate thick samples (Singh and Gräfe 2010).

2.7. Summary of Literature Review

Shober et al. (2006) characterized phosphorus speciation in manures and biosolids using the XANES, and they found that one extractant might extract either part or some combination of P species. Li et al.(2014) characterized the P speciation of Chesapeake Bay sediments using the chemical sequential extraction (CSE), ^{31}P NMR, and EXAFS spectroscopy. They pointed out that the citrate-dithionite-bicarbonate extraction could represent more P pools than those P species classically presumed. However, both Shober et al. (2006) and Li et al. (2014) did not further study the mechanism of CSE method, because they did not identify the P species extracted by each extractant and the P species left in the residue of each fraction. Therefore, there is a knowledge gap for understanding the functions of the CSE method.

Lukkari et al. (2007) investigated the reproducibility of the Jensen and Thamdrup (1993) method and variations within the extracts obtained at each step with a commercial reference material. The research showed good reproducibility of the Jensen and Thamdrup (1993) method. And the

method gave reliable results for identifying the potentially mobile and immobile P in sediments. However, Lukkari et al. (2007) did not use XANES nor ^{31}P NMR to study the molecular characteristics of P in each fraction of the CSE.

Kar et al. (2011) compared the speciation of P in soils amends as using a combination of CSE and XANES on the solid-phase residues after each extraction step. Kar et al. (2011) did not investigate the P speciation in the supernatant of each extraction step. Also, it lacked the information about organic P in the residue because XANES has limited functions in determining the organic P species. In addition, Kraal et al (2015) found that using the XANES to distinguishing between various organic P phases is challenging. They pointed out the need of using ^{31}P NMR for characterization of the organic P speciation.

Ajiboye (2007) attempted to identify P species in each step of the sequential extraction procedure using the XANES and ^{31}P NMR. However, the sequential extraction procedure is used for studying the P species in organic amendments. Thus, another knowledge gap is the lack knowledge of CSE in its function to determine the P fractionation in the lake sediments.

To summarize, the main objective of this study is to enhance the understanding of the Jensen and Thamdrup (1993) chemical sequential extraction method for studying the sedimentary phosphorus fractionation by using solution ^{31}P NMR spectroscopy and phosphorus K-edge XANES spectroscopy.

The sub-objectives for this study are the following:

- To study P fractionation in the lake sediments using the CSE, XANES and ^{31}P NMR;
- To characterize the mineral P species in the supernatant and residue in each step of the CSE using the XANES;
- To identify the organic P species in the supernatant and residue in each step of the CSE using the ^{31}P NMR.

CHAPTER 3 Materials and Methods

3.1. Sediments Sampling

A total of four sediments samples were collected by sediments cores (length 36cm, diameter 5cm, coextruded PVC tubes) from Blackstrap Lake and Royal Canadian Air Force (R.C.A.F) Pond in Saskatchewan. The sediments cores were immediately frozen in a freezer after sample collection.

Blackstrap Lake is the largest reservoir in the Saskatoon South East Water Supply System (City of Saskatoon). The Primary inflow is gravity fed via an earthen aqueduct from Lake Diefenbaker. Blackstrap Lake is developed as a result of the construction of Gardiner Dam. It is located 4.8 km east of the village of Dundurn in a post-glacial river valley. It is about 14.4 km long and varies from 0.8 to 1.2 km wide. Water was first released into the reservoir in July 1967, and it was filled by November of the same year. The water covers a surface area of 3,000 acres (1,200 ha). The average depth is about 5.15 m with a maximum depth of 9.39 m. The reservoir is fulfilling its basic function as a holding reservoir for irrigation, industry, and municipal supply. In addition, extensive recreational facilities have been provided at Blackstrap Lake (Hwang et al. 1975).

Blackstrap #3 and Blackstrap #6 are the names of two sediments samples that were collected from Blackstrap Lake. The map of Blackstrap Lake and the locations of the samples are shown in Figure 3.1. The location of Blackstrap #3 was described as 51°46'18.26"N and 106°27'39.14"W, and Blackstrap #6 was collected at 51°46'34.79"N and 106°27'36.20"W.

Royal Canadian Air Force (R.C.A.F) Pond is one of the storm water ponds in City of Saskatoon, Saskatchewan. A storm water retention pond is an engineered artificial body of water. Its primary function is to protect residential properties from flooding by storing peak storm water flow and street run-off and releasing it into the storm sewer collection system in a controlled manner. This pond is an important part of the City's storm water management system (City of Saskatoon).

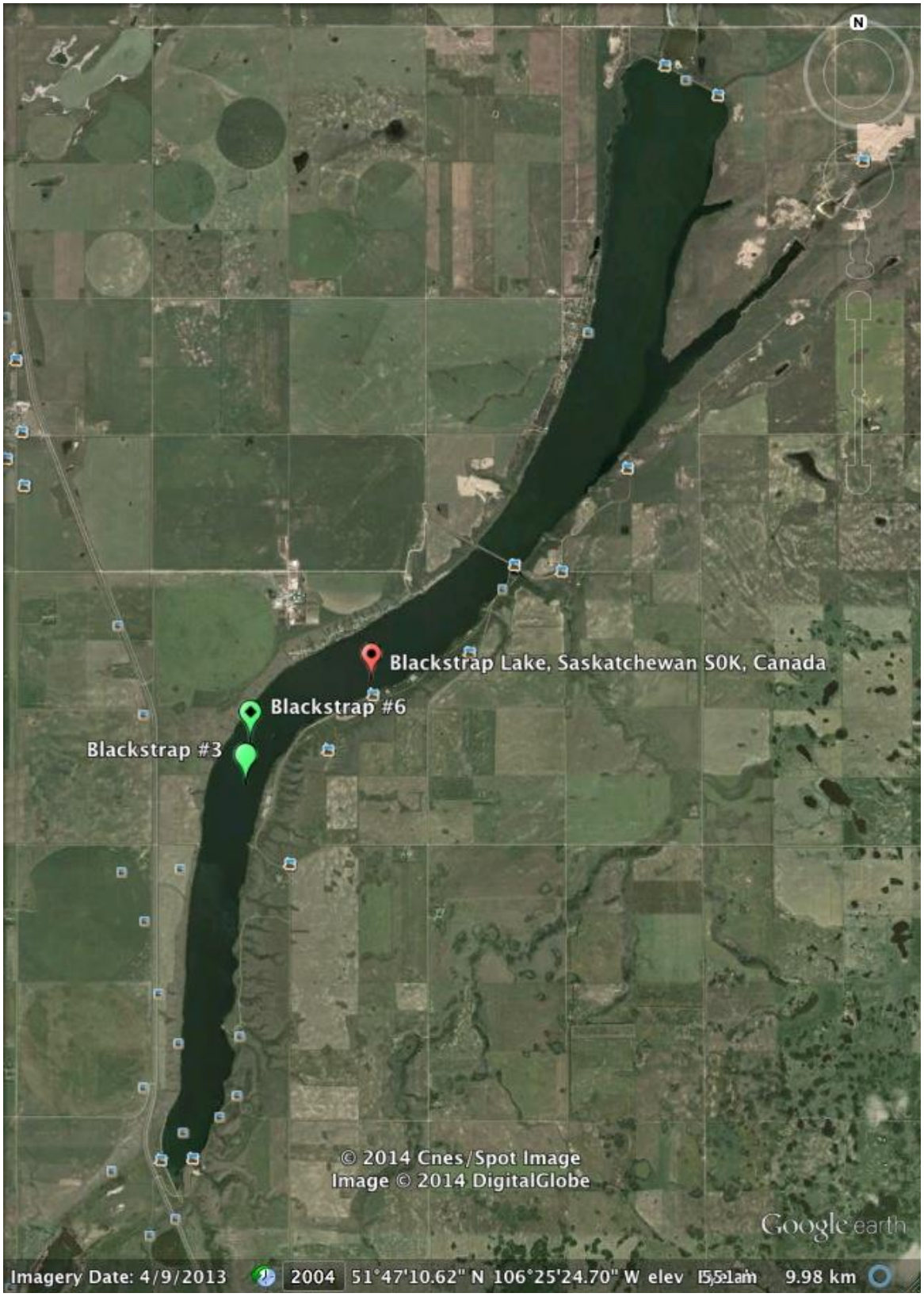

Figure 3.1. Site plan showing locations of Blackstrap Lake and samples

Pond #3 and Pond #11 are the names of two sediments samples that were collected from R.C.A.F Pond. The map of R.C.A.F Pond and the samples locations are shown in Figure 3.2. Pond #3 was collected at 52° 9'57.17"N and 106°40'18.57"W, and the location of Pond #11 was described as 52° 9'57.26"N and 106°40'20.26"W.

3.2. Water Contents and Volatile Solids of Sediments Samples

Each lake sediment sample was completely mixed well. Approximately 5 grams of each well-mixed sample was used to measure the water contents. Water contents of the lake sediments samples were estimated by weight losses following overnight drying at ~105ºC within Canlab oven (made by LABLINE. Inc. Chicago, ILL USA; Category number: 30505K) (Heiri 2001).

Water loss was estimated as the weight difference between wet sample weight and sample weight following overnight drying at ~105ºC (Equation 3.1 and Equation 3.2):

$$Water\ Loss\ (g) = Sample\ Wet\ Weight - Sample\ Dry\ Weigh \quad [3.1]$$

Water contents were estimated as water loss divided by wet sample weight (Equation 3.2).

$$Water\ Contents(\%) = \frac{Water\ Loss}{Sample\ Wet\ Weight} \times 100\% \quad [3.2]$$

Volatile solids were readily calculated then as the difference in weight between the sediments dried at 105ºC and the ash created by being ignited at 550ºC for 2 hours within muffle furnace (Heiri 2001). The percentage of volatile solids was calculated by Equation 3.3.

$$Volatile\ solids\ (\%) = \frac{Weight\ Post\ 105℃\ Dry\ Sample - Weight\ of\ Post\ 550℃\ Ash}{Weight\ Post\ 105℃\ Dry\ Sample} \times 100\% \quad [3.3]$$

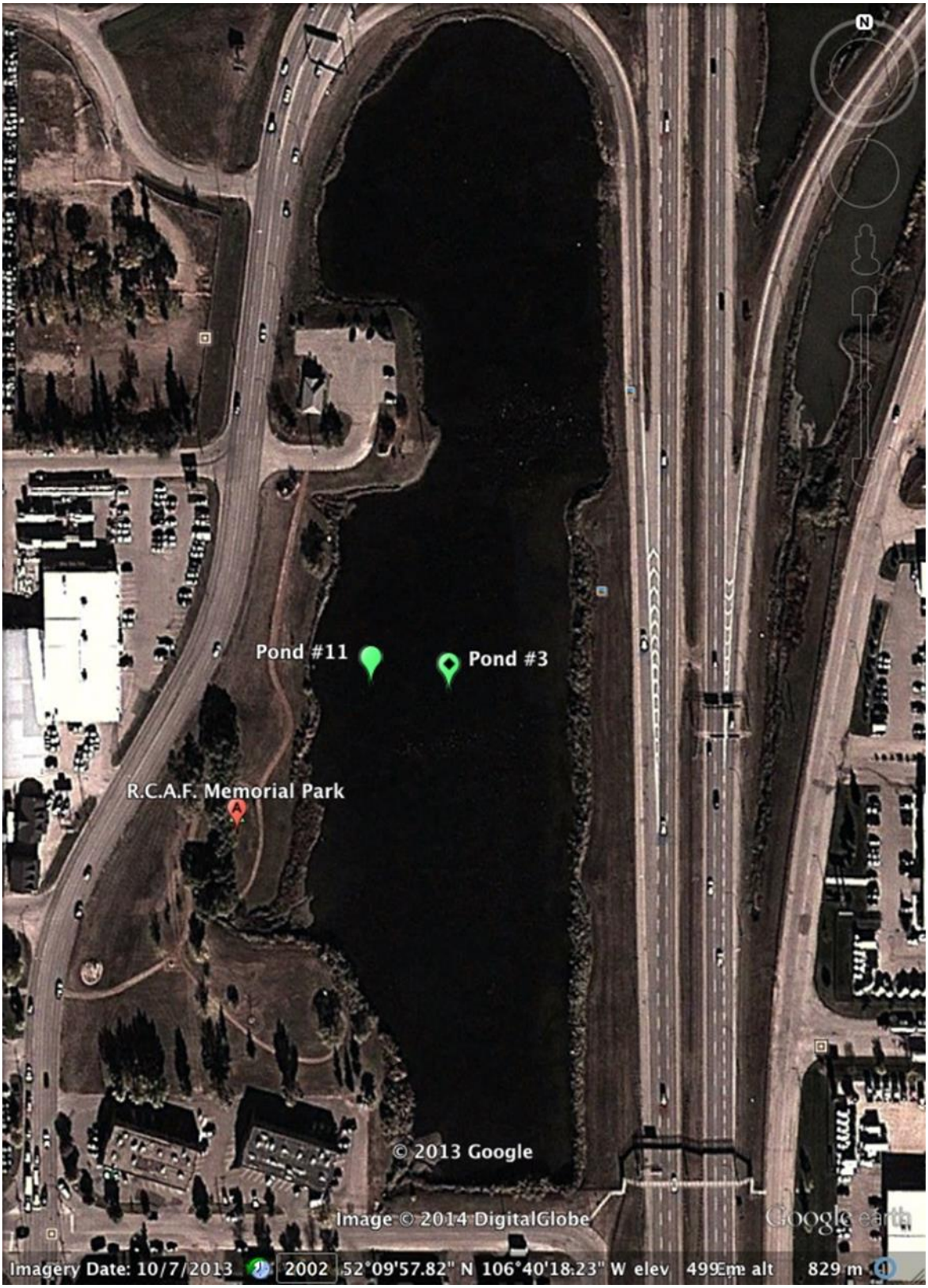

Figure 3.2. Site plan showing locations of R.C.A.F Pond and samples

3.3. Sequential Phosphorus Extraction Analysis

Chemical sequential extraction (Jensen and Thamdrup 1993) was used in this study. Approximately 1 gram of well-mixed wet sediments sample was placed into a 40 mL polyethylene centrifuge tube. Different fractions of P in the sediments were extracted according to the scheme described in Figure 3.3. Samples were centrifuged for 10 min at 3,000 rpm. The supernatants were filtered through a 0.45-μm membrane filter. Soluble reactive phosphorus (SRP) in the supernatants was analyzed by the ascorbic acid method (4500-P E. Rice et al. 2012). Total phosphorus (TP) in the supernatants after persulfate digestion was measured with the ascorbic acid method. The non-reactive phosphorus (NRP) in each supernatant was calculated by subtracting the determined SRP concentration from the determined total phosphorus (TP) concentration (4500-P E. Rice et al. 2012).

Step 1 was supposed to extract the pool of loosely adsorbed phosphorus (labile-P), pore-water SRP and pore-water NRP. In step 1, 1 mL of 1 M H_2SO_4 was added to the tubes to prevent co-precipitation of phosphate with iron (Fe) and manganese (Mn) and to preserve the sample.

In step 2, the bicarbonate dithionite solution (BD-reagent) was created by adding 0.11 M $Na_2S_2O_4$ to the stock solution of 0.11 M $NaHCO_3$ in a 1:1 mix. After adding 8mL 1 M H_2SO_4 to avoid precipitation of Fe and Mn, the following 1-hour aeration was intended to oxidize the remaining dithionite. The addition of 1 M H_2SO_4 and 1 hour aeration led to the formation of elemental sulfur, which turned the solution milky. Normally, the sulfur precipitated within few days and then the clear solution was tested for SRP. Step 2 was designed to primarily extract biological available phosphorus (Fe-bound P).

In step 3, 0.1 M NaOH was added to the residue from step 2 to extract SRP from clay minerals and aluminum oxides (Al-bound P) together with organic phosphorus which appears as NRP in the solution. Three milliliters of 1 M H_2SO_4 was added to the tubes to prevent co-precipitation of phosphate with Fe and Mn and to preserve the sample. In step 4, 0.5 M HCl was added to the residue from step 3 to extract calcium-bound P such as apatite.

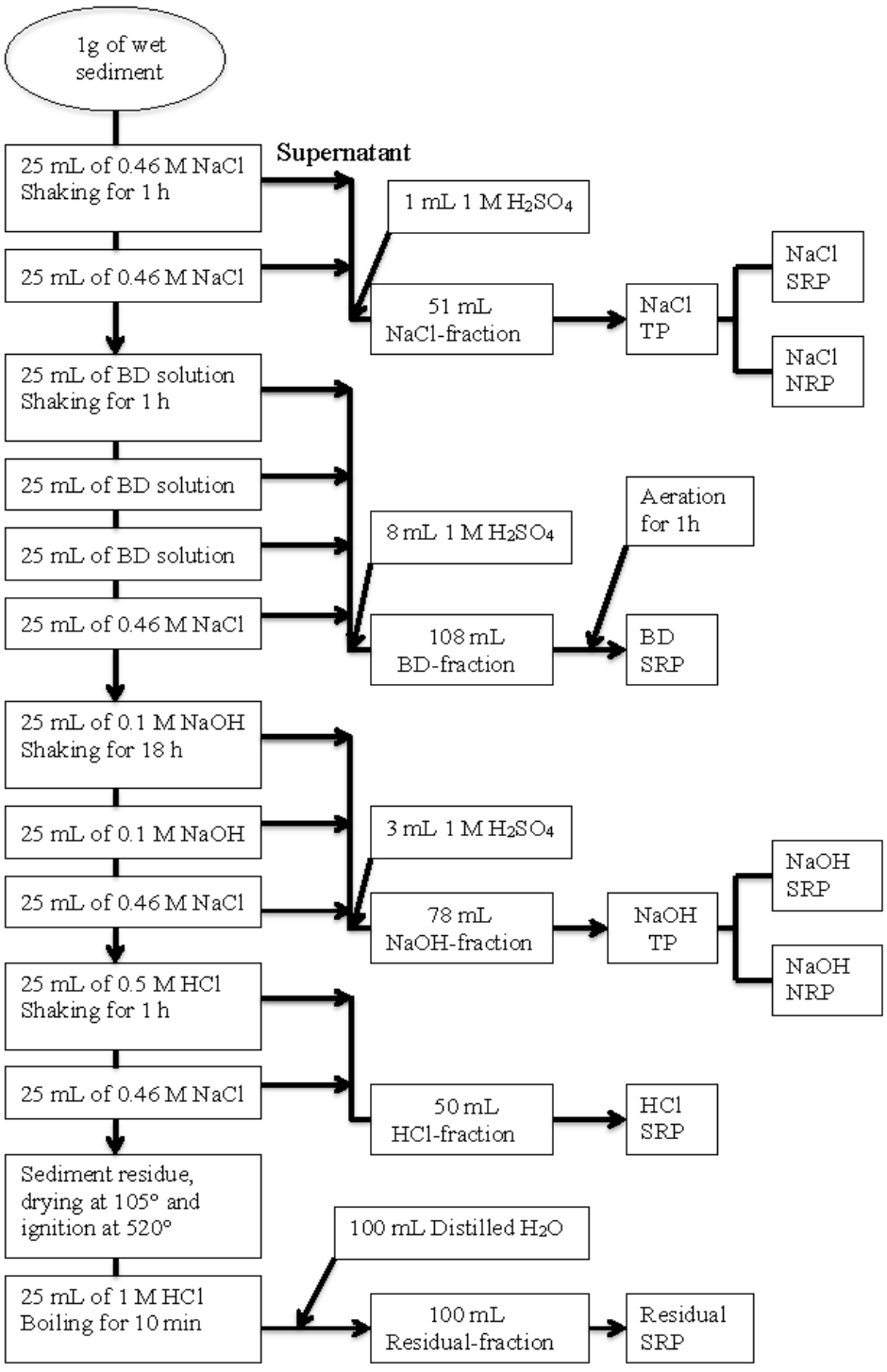

Figure 3.3. Sequential extraction scheme for phosphorus fractions in the sediments (Modified from Jensen and Thamdrup 1993)

In the last step the sediments pellet was ignited at 520 °C for two hours and subsequently boiled for 10 minutes in 1 M HCl. The residual phosphorus left in the sediments was extracted. Residue P represented the more refractory organic P pool (Jensen and Thamdrup 1993).

3.4. Acid-Digestion Analysis for Total Phosphorus in the Sediments

Total phosphorus in the sediments was determined by the modification of total phosphorus Parkinson and Allen Digestion method proposed by Cade-Menun and Lavkulich (1997). For these measurements, 2 grams of wet well-mixed sediments sample was transferred into a 250 mL digestion tube made of glass, 12.5 mL of 98% H_2SO_4 was added into each tube, and afterwards the sample and the 98% H_2SO_4 were mixed on the vortex mixer immediately. Then 2.5 mL of Li_2SO_4 solution was added to each tube causing a chemical reaction. After the reaction finished (approximately 5 minutes), a second 2.5 mL increment of Li_2SO_4 solution was added to each tube and was allowed to settle until the reaction was finished (approximately 5 minutes). When digestion block was heated up to about 360℃, the rack of tubes was heated on the block using intermittent heating.

After the samples remained on the block for at least 5 minutes without excessive spattering, they were allowed to digest for 1.5 hours. After 1.5 hours, the samples were removed from block. After cooling for 5-10 minutes, 2.5 ml of H_2O_2 was added. Samples were put back on block for an hour digestion.

After total 2.5 hours digestion, samples were removed from block. Samples were allowed to sit overnight, and deionized water was added to reach a volume of 250 mL before colorimetric analysis. Colorimetric analyses of P in these extracts were analyzed by the ascorbic acid method (4500-P E; Rice et al. 2012).

3.5. Solution ^{31}P NMR Spectroscopy Analysis

The extractant used was 0.5 M NaOH plus 0.1 M Na_2EDTA in a 1:1 mix. Fifteen grams of well-mixed wet sediments sample was placed into 50 mL of extractant. All samples were extracted in 125 mL Erlenmeyer flasks at room temperature overnight with stirring. Samples were then centrifuged at 15000 rpm for 20 min. And then samples were filtered with Buchner funnels and

Whatman 41 filter paper (Maidstone, UK). The filtrates of samples were freeze-dried by Labconco Freezone freeze dryer (Cade-Menun et al. 2002).

To prepare the samples for NMR spectroscopy, 0.3 to 1.0 grams of the freeze-dried supernatant was added into a 50 mL plastic centrifuge tube with 2.6 mL of D_2O and 0.4 mL of 10 M NaOH. The NaOH solution was added here to increase the pH of samples to 12 for optimal peak separation (Crouse et al. 2000). Samples were vortexed for 2 min, centrifuged for 20 min at 3000 rpm, and decanted into 10-mm NMR tubes. The samples were prepared no more than one hour prior to analysis by NMR spectroscopy. Solution ^{31}P NMR spectra were acquired at 202.45 MHz on a Bruker DRX-500 spectrometer running XWIN-NMR v2.1 and equipped with a 10-mm broadband observation probe.

The end result of an NMR experiment is the emission signal, which was detected and recorded as free-induction decay (FID). A FID is a time domain signal because it is recorded as a function of intensity over time. It is converted by Fourier transformation using the processing software to a frequency domain spectrum, with signal intensity plotted as a function of frequency. All spectral data analysis was carry out with SpinWorks 3.1.8 processing software. Interleaved FIDs were separated and co-added with standard automation programs. Spectra were processed with a line-broadening of 7 Hz. All the spectra were calibrated by setting the intense peak of the inorganic orthophosphate to 6.0 ppm. Subsequently, the peaks in the calibrated spectra were assigned to P species and organic P functional groups according to the literature values. (Turner and Richardson 2004; Cade-Menun 2005)

Peak-picking, peak-intensity determination, and spectral integration were performed with automated peak analysis tools. Following the method of Cade-Menun (2005), the entire spectrum was integrated, and the integral was then divided into regions representing each peak using an integration routine in the processing software. After printing the spectrum and integral, the height of the integral for each peak was measured manually with a ruler, and the proportion as a percent of the total height of the integral was determined, which was the percent of total sample P in each P species. Processing software may also be used to determine the value of the integral for each peak, but this only works well for spectra with high signal to noise ratio (S/N) and high spectral resolution (Cade-Menun 2005).

In previous study, it shown the standard errors associated with estimating the proportion of P species by peak integration were approximately 5% and 10% for large and small peaks respectively (Leinweber et al. 1997).

3.6. Phosphorus K-edge XANES Spectroscopy Analysis

Phosphorus K-edge XANES was carried out at the Canadian Light Source's Soft X-ray Microcharacterization Beamline (SXRMB) 06B1-1. Figure 3.4 shows the schematic layout of the beamline. The beamline was calibrated for P K-edge by setting the white line (due to P 1s – 3p transition) of sodium pyrophosphate at 2152.4 eV on the energy scale (Lombi et al. 2006).

P K-edge XANES was conducted on freeze-dried sediments samples, some of the chemical sequential extractions supernatants and some of the residues after chemical sequential extraction. The freeze-dried samples were ground to powder, thinly spread over conducting carbon tape and mounted on a stainless steel sample holder. A variety of phosphate standards that were used in this study included apatite, aluminum phosphate, and phytic acid (Table 3.1).

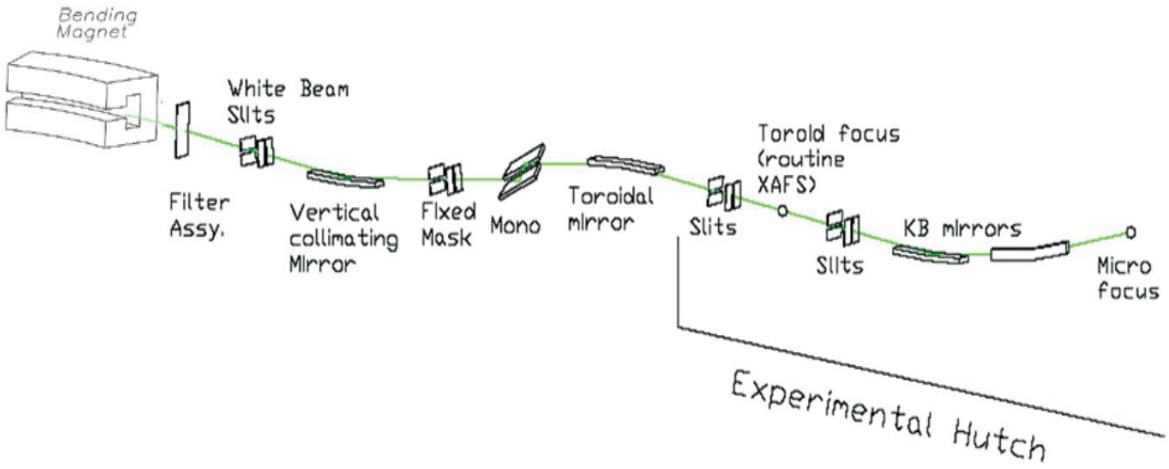

Figure 3.4. Schematic layout of the SXRMB (Adapted from http://www.lightsource.ca/uso/pdf/06B1-1_SXRMB_Beamline_Characteristics.pdf)

Table 3.1. Inorganic and organic reference P compounds used in the experiment

Reference compound	Chemical formula
Apatite	$Ca5(PO_4)_3(OH,F,Cl)$
Aluminum phosphate	$AlPO_4$
Phytic acid	$C_6H_{18}O_{24}P_6$

Data was collected at the P K-edge (2152 eV) in fluorescence mode using a 4 element solid-state Si (Li) detector tuned to the P edge. Spectra were collected from 2131 to 2190 eV in 0.15 eV steps. Multiple scans were averaged to obtain acceptable signal-to-noise ratios for spectral analysis. Data analysis was performed in OriginLab 9.0. A linear base line was fit through the pre-edge region 2140 to 2150 eV of the averaged XANES files. And the spectra were normalized through the post-edge region 2180 to 2190 eV of the averaged XANES files. Qualitative P K-edge XANES analysis was carried out using fingerprinting approach in identifying the P species in the sediments.

CHAPTER 4 Results and Discussion

4.1. Water Contents, Volatile Solids and Total Phosphorus of Lake Sediments Samples

The water contents and volatile solids in the sediments samples are shown in Table 4.1. From the table, the water contents and the percentages of volatile solids of Pond #3 and Pond #11 are larger than those of Blackstrap #3 and Blackstrap #6. The reason that sediments from pond have a larger amount of volatile solids is that the sampling positions of Pond #3 and Pond #11 were close to the storm sewer inlet, which could bring organic matters into the Pond.

Total phosphorus (TP) in the sediments are shown in Table 4.2. Total phosphorus was determined by the modification of total phosphorus Parkinson and Allen Digestion method proposed by Cade-Menun and Lavkulich (1997). The TP in the sediments sample Blackstrap #3, Blackstrap #6, Pond #3 and Pond #11 were 474 mg/kg, 519 mg/kg, 684 mg/kg and 729 mg/kg, respectively. The TP concentrations in sediments samples from Pond are larger than those in sediments samples from Blackstrap.

Table 4.1. Water contents and volatile solids (Mean ± Standard Error)

Sediments sample	Sediment core length (cm)	Water contents (%)	Volatile solids (%)
Blackstrap #3	15	41.29 ± 4.40	4.33 ± 1.17
Blackstrap #6	18	34.70 ± 0.09	2.32 ± 0.07
Pond #3	10	71.34 ± 4.39	19.10 ± 1.04
Pond #11	12	67.93 ± 0.95	20.19 ± 0.32

4.2. Phosphorus Fractionation in Lake Sediments Samples

The results of the chemical sequential extraction on four sediments samples are shown in Table 4.2. First of all, the amounts of total phosphorus in Pond #3 and Pond #11 are larger than those in Blackstrap #3 and Blackstrap #6. The inorganic P was predominated in the sediments samples, which accounted for 79.68%, 90.88%, 73.84% and 68.43% of total phosphorus respectively in Blackstrap #3, Blackstrap #6, Pond #3, and Pond #11.

Also, from Table 4.2, the total phosphorus obtained by chemical sequential extraction was smaller than the total phosphorus determined by acid digestion. Parkinson and Allen digestion is a good method for total P determination because it extracts completely total P from mineral soils (Cade-Menun and Lavkulich 1997). Besides, the standard error of NaOH-NRP was higher than those of other fractions. When H_2SO_4 was added to NaOH extracts, brownish precipitate was formed. Thus, it interfered the spectrophotometric analysis of phosphorus.

Table 4.2. Sequential phosphorus forms extracted in the sediments (Mean ± Standard Error)

	Blackstrap #3	Blackstrap #6	Pond #3	Pond #11
P Pool	P concentration (mg/kg)			
Inorganic P				
NaCl-SRP	4.31 ± 0.43	1.99 ± 0.39	3.16 ± 0.36	5.21 ± 0.00
BD-SRP	45.07 ± 1.86	47.36 ± 0.05	110.18 ± 2.73	94.24 ± 1.78
NaOH-SRP	23.50 ± 0.06	17.31 ± 0.83	48.88 ± 3.45	40.25 ± 3.59
HCl-SRP	272.32 ± 6.91	304.80± 7.18	217.27 ± 2.05	200.57 ± 1.25
Inorganic P	345.19 ± 7.17	371.44 ± 7.24	379.49 ± 4.87	340.26 ± 4.20
Organic P				
NaCl-NRP	4.01 ± 0.98	5.42 ± 0.71	5.33 ± 1.05	3.15 ± 0.01
BD-NRP	nd	nd	nd	nd
NaOH-NRP	53.13± 26.71	8.18 ± 1.12	94.44± 22.46	122.74± 41.26
HCl-NRP	nd	nd	nd	nd
Organic P	57.06± 26.73	13.60 ± 1.32	99.77 ± 22.48	125.89 ± 41.26
Residual P	30.99 ± 3.92	23.67 ± 1.22	34.66 ± 2.47	31.12 ± 5.51
Total P(CSE)	433.24 ± 27.95	408.72 ± 7.46	513.91 ± 23.14	497.27 ± 41.84
Total P(Digestion)	474.06 ± 9.41	518.90 ± 10.36	684.17 ± 7.11	728.53 ± 5.38

Based on the chemical sequential extraction analysis, phosphorus concentrations in different fractions of sediments samples are shown in Figure 4.1, and percentage composition of total phosphorus in sediments samples are shown in Figure 4.2. The types of phosphorus species that the legends in Figure 4.1 and 4.2 represent are listed in Table 4.3. These types are defined by the Thamdrup and Jensen (1993) chemical sequential extraction method. Ca-P is the largest P fraction of every sediments sample, which exhibited great difference between the sediments samples from Blackstrap (62.86% to 74.57%) and sediments samples from Pond (42.28% to 40.33%). Ca-P is a stable fraction of sedimentary P, which is not easily released from sediments.

The second largest fraction is Fe-P or Organic-P. The amount of Fe-P varied among the samples as follows: 10.40%, 11.85%, 21.17%, and 18.95% respectively. Fe-P is a potentially mobile P, which is sensitive to low redox potentials. Phosphate moves from sediments into the overlying water when the sediments surface becomes anaerobic, because ferric is changed to ferrous (Mortimer 1942). Organic-P may include bacteria-incorporated P, DNA and polyphosphates. Labile P, also called loosely adsorbed P, is the smallest fraction in every sediments sample, which was around 1%.

It is necessary to discuss the related P sources for Blackstrap Lake and R.C.A.F pond. For Blackstrap Lake, the possible P sources include nutrients regeneration from the sediments, agriculture runoff (inorganic fertilizer), farm runoff (private waste disposal systems, animal manure, animal feedlots, farm wastewater), and wastewater from the residents and visitors of Blackstrap Provincial Park. R.C.A.F pond is located nearby the highway and industrial communities, and has three storm sewer inlets. The possible P sources for the pond are urban runoff though piped sewer systems and effluents from industrial treatment facilities.

Table 4.3. Legends of Figure 4.1 and Figure 4.2

Legend	Type of phosphorus compounds
NaCl-SRP	Labile-P
NaCl-NRP	Pore-water NRP
BD-SRP	Fe-P
NaOH-SRP	Al-P
NaOH-NRP	Organic-P
HCl-SRP	Ca-P
Residue P	Refractory P

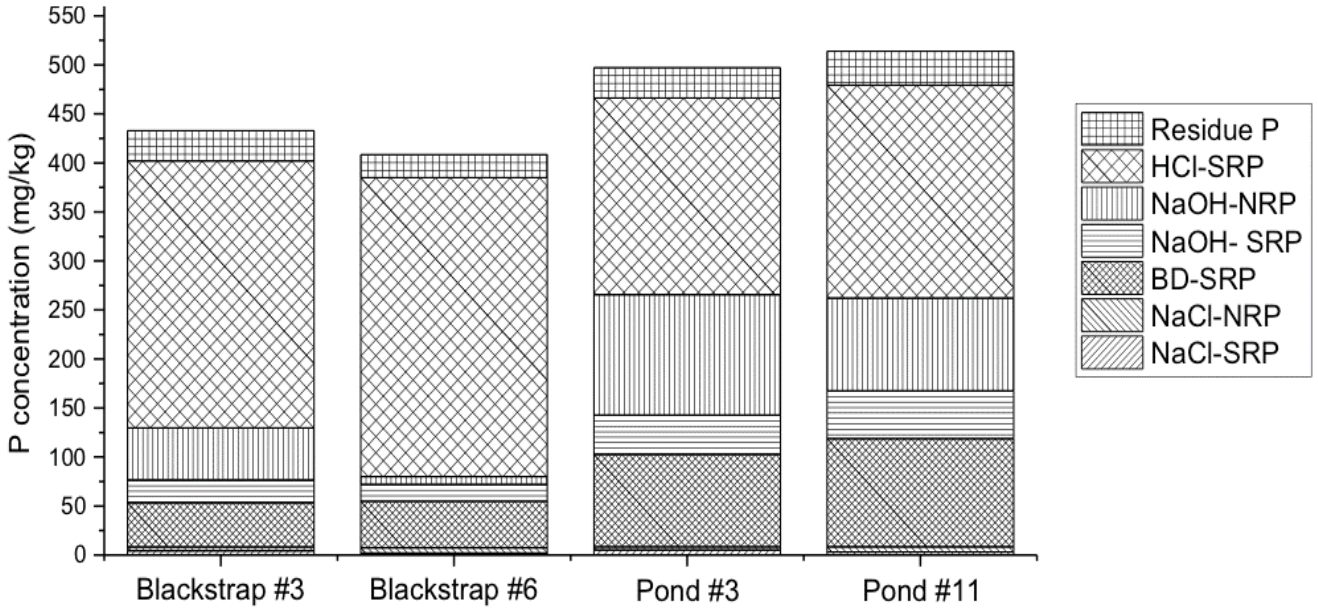

Figure 4.1. Phosphorus concentrations in different fractions of the sediments samples

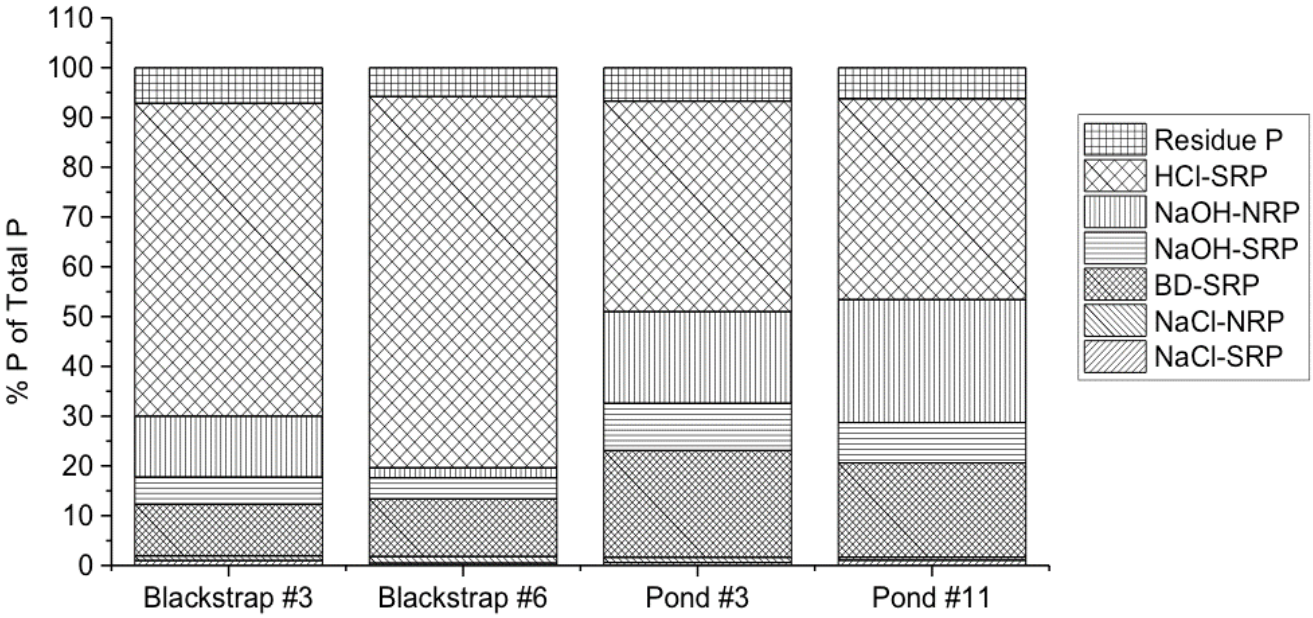

Figure 4.2. Percentage composition of total phosphorus in the sediments samples

4.3. Solution ^{31}P NMR Spectra Interpretation

4.3.1. Identification of Phosphorus Species in NaOH-EDTA extracts by ^{31}P NMR

The solution ^{31}P NMR spectra of sediments samples and reference sample are shown in Figure 4.3 to Figure 4.7. The X-axis in the figures is chemical shift, and the Y-axis is the spectral resolution. The chemical shifts of the spectra ranged from -25 ppm to 25 ppm where the phosphorus forms of interest in environmental studies fall between (Cade-Menun 2005). According to Cade-Menun, the spectra were separated into four regions in order to assign the peaks. The four regions were 25 ppm to 7 ppm (phosphonates), 7 ppm to 2.5 ppm (orthophosphate monoesters), 2.5 ppm to -4.4 ppm (orthophosphate diesters) and -4.4 ppm to -25 ppm (polyphosphate end groups) (Cade-Menun 2005). There were no significant peaks observed between 25 ppm to 7 ppm and -4.5 ppm to -25 ppm for the four sediments samples collected, thus the regions of 7 ppm to 2.5 ppm and 2.5 ppm to -4.5 ppm were zoomed in the Figure 4.3 to Figure 4.7.

For Blackstrap #3, the assignments of the peak in the ^{31}P NMR spectra (Figure 4.3) were shown as follows. Chemical shift (ppm) of the peak at 6 ppm was assigned to orthophosphate. Peaks at 5.68, 4.80, 4.75 and 4.50 ppm in the ration 1:2:2:1 were assigned to myo-inositol hexakisphosphate (phytic acid) (Cade-Menun 2005). The peak at -0.55 ppm was assigned to deoxyribonucleic acid (DNA) and the other peak at -4.19 ppm was assigned to pyrophosphate (Cade-Menun 2005). Other peaks could not be assigned to any specific P species, based on the library of chemical shifts of model P compounds in Table 2.1.

The spectra of Blackstrap #6 are shown in Figure 4.4. Peak at 6 ppm was assigned to orthophosphate. Peaks at 5.68, 4.52, 4.32, and 4.20 ppm were assigned to phytic acid (Cade-Menun 2005), while those at -0.62 and -4.25 ppm were assigned to DNA and pyrophosphate, respectively.

To confirm the assignment of peaks of phytic acid, spiking was performed. The spectra of spiking of Pond #3 and phytic aid are shown in Figure 4.5. It can be seen that four peaks were shown at approximately 5.66, 4.69, 4.32 and 4.23 ppm, respectively. These peaks were in the ratio of 1:2:2:1 corresponding to P_2: P_4+P_5:P_1+P_3:P_6 on the inositol ring which is the structure of

phytic acid. The chemical shift of peaks at 5.68, 4.52, 4.32, and 4.20 ppm of the spectra of Blackstrap #6 were close to that of the spectra of spiking. Therefore, it is correct to assign those peaks to phytic acid.

For Pond #3, the spectra are shown in Figure 4.6. Chemical shift of the peak at 6 ppm was assigned to orthophosphate. Peaks at 5.66, 4.69, 4.32, 4.23 ppm in the ratio of 1:2:2:1 were assigned to phytic acid, while those peaks at -0.50, -3.90, and -4.18 ppm were assigned to DNA (Cade-Menun 2005), polyphosphate end group and pyrophosphate, respectively.

The assignments of peak in the spectra of Pond #11 (Figure 4.7) were shown as follows. Peak at 6 ppm was assigned to orthophosphate. Peaks at 5.68, 4.60, 4.32, 4.25 ppm were assigned to phytic acid, while those peaks at -0.50, -3.90, and -4.18 ppm were assigned to DNA, polyphosphate end group and pyrophosphate, respectively.

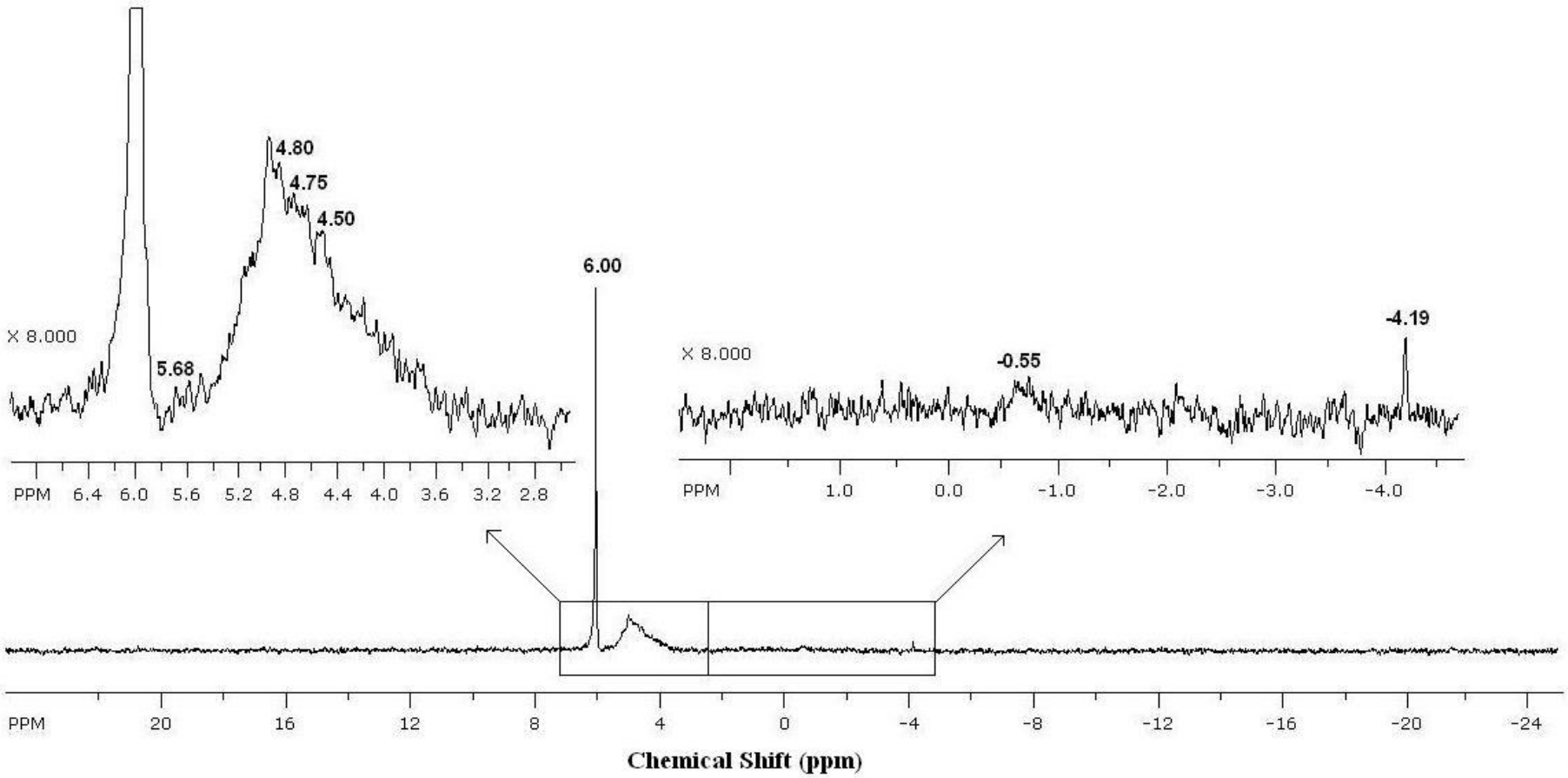

Figure 4.3. ^{31}P NMR spectra of Blackstrap #3 extracted with NaOH–EDTA. Zoomed in (7ppm to 2.5ppm) and (2.5ppm to -4.5ppm)

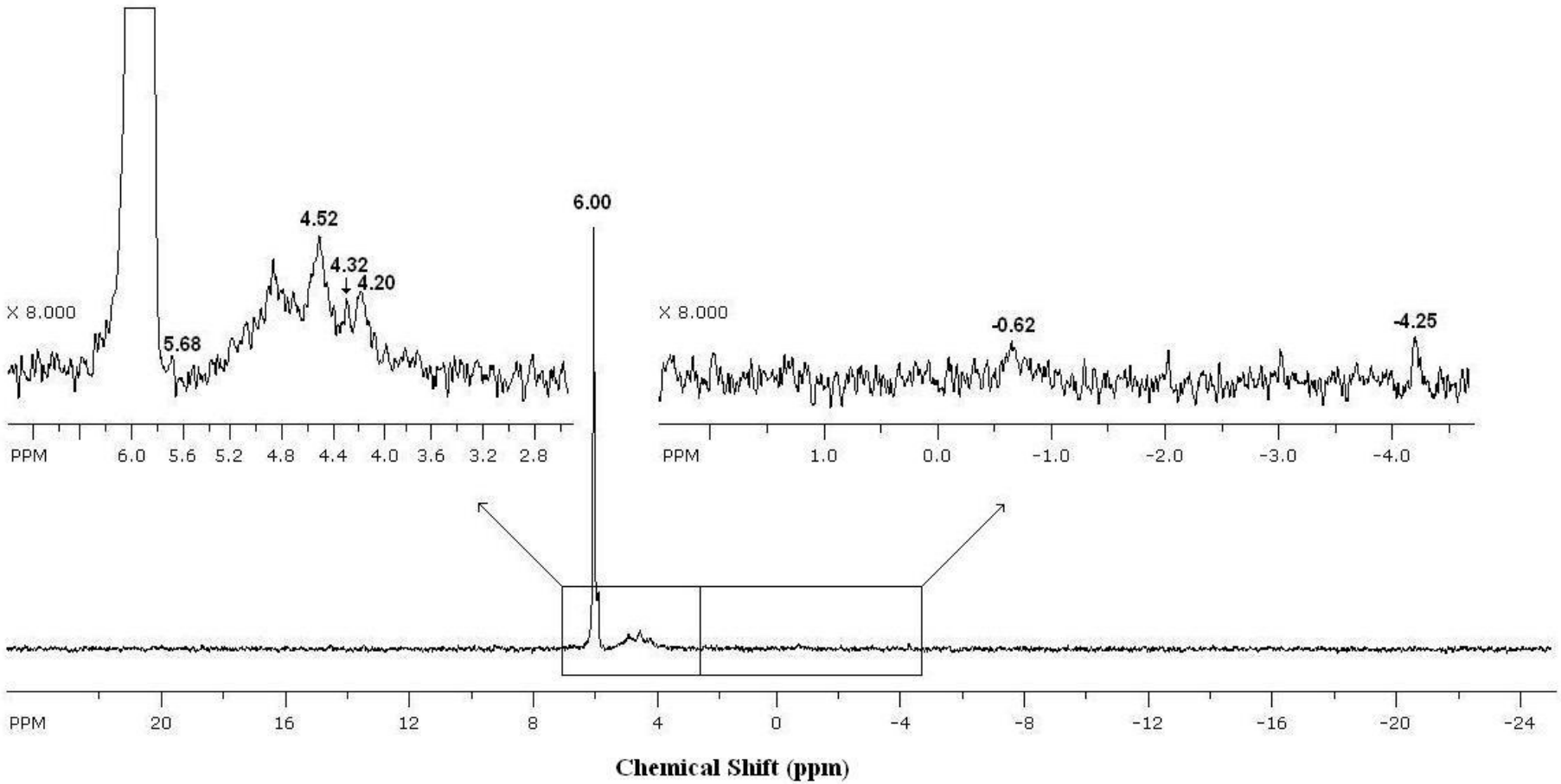

Figure 4.4. ^{31}P NMR spectra of Blackstrap #6 extracted with NaOH–EDTA. Zoomed in (7ppm to 2.5ppm) and (2.5ppm to -4.5ppm)

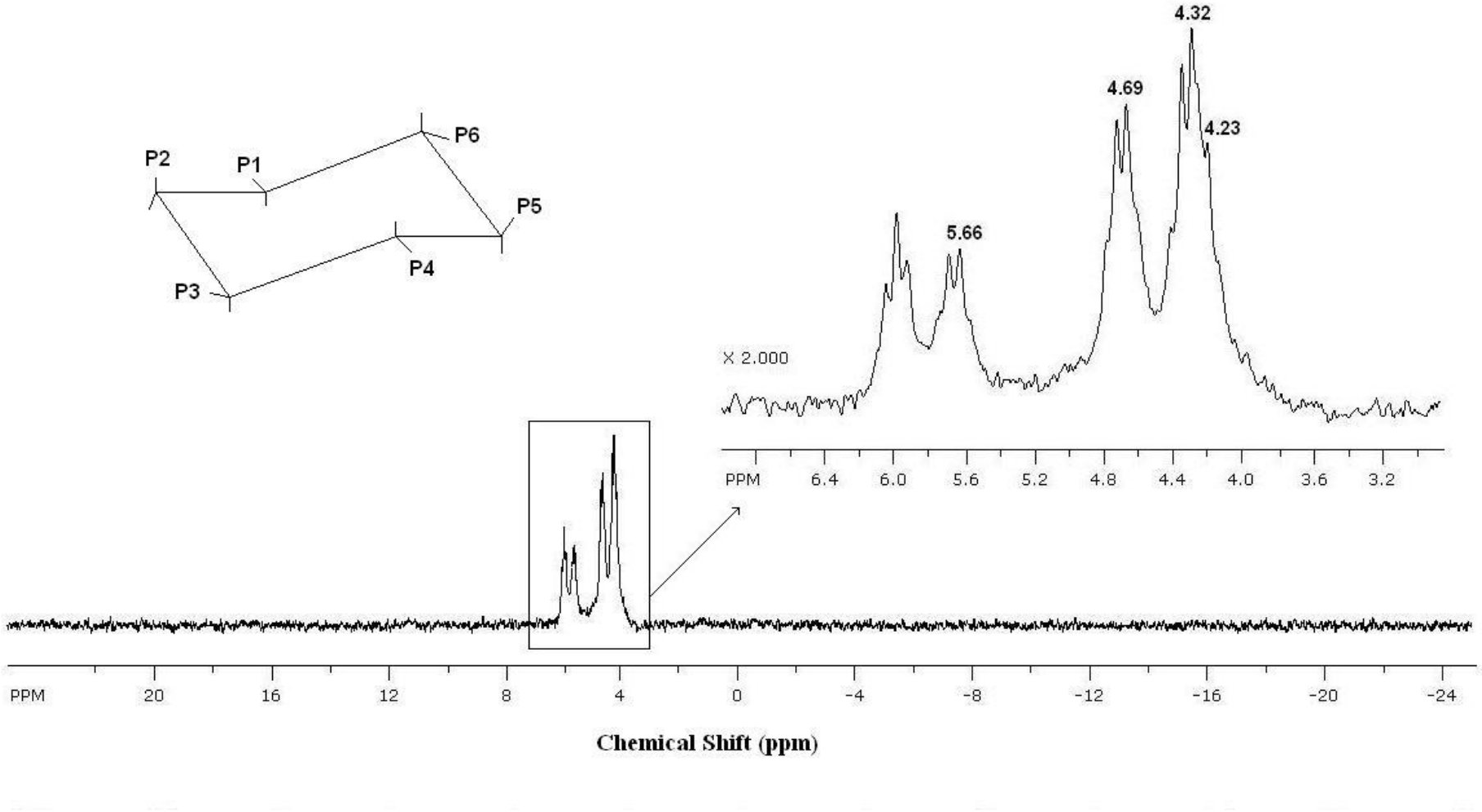

Figure 4.5. ^{31}P NMR spectra of spiking myo-inositol hexakisphosphate (phytic acid). Zoomed in (7 ppm to 2.5 ppm)

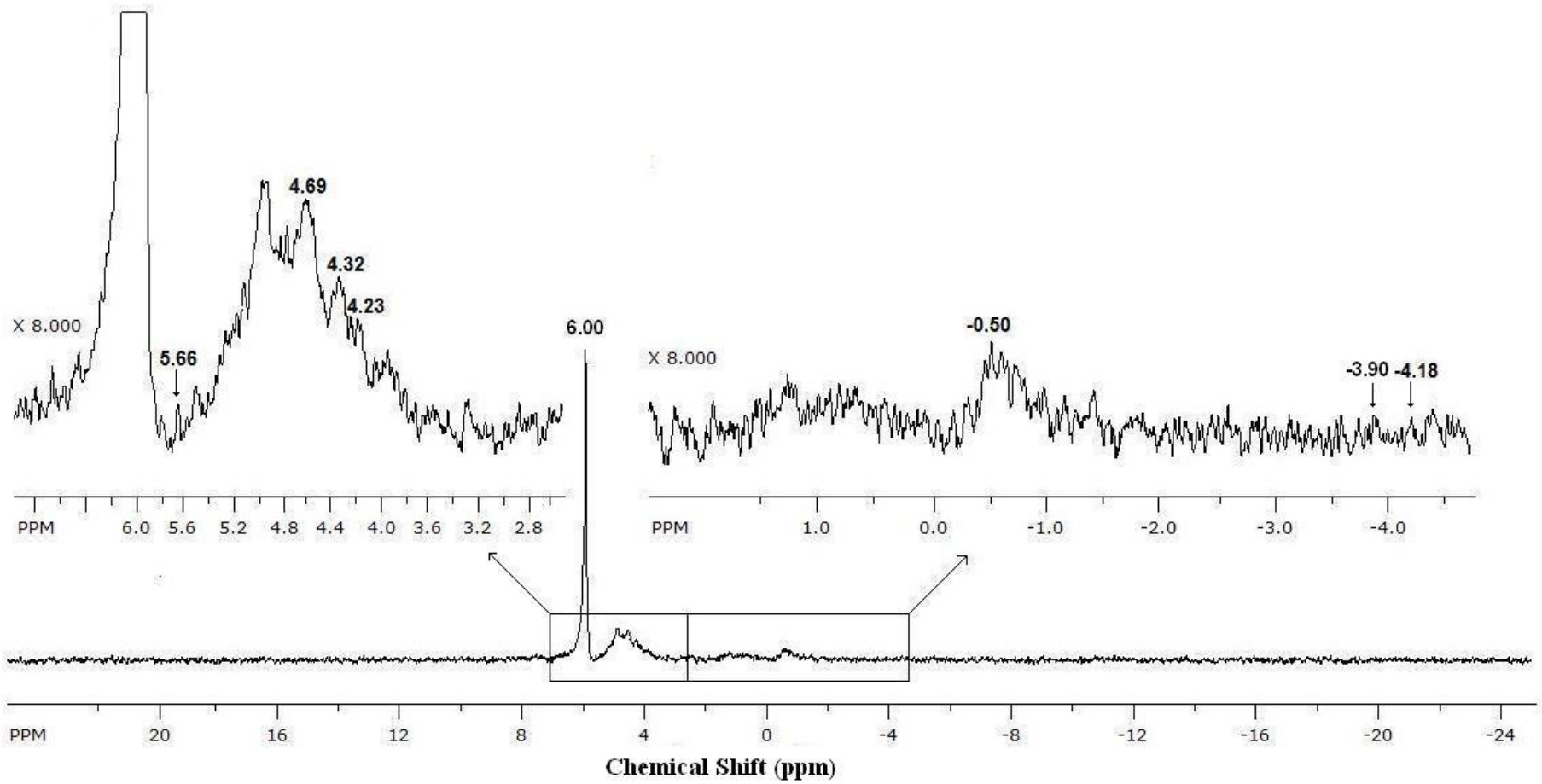

Figure 4.6. ^{31}P NMR spectra of Pond #3 extracted with NaOH–EDTA. Zoomed in (7ppm to 2.5ppm) and (2.5ppm to -4.5ppm)

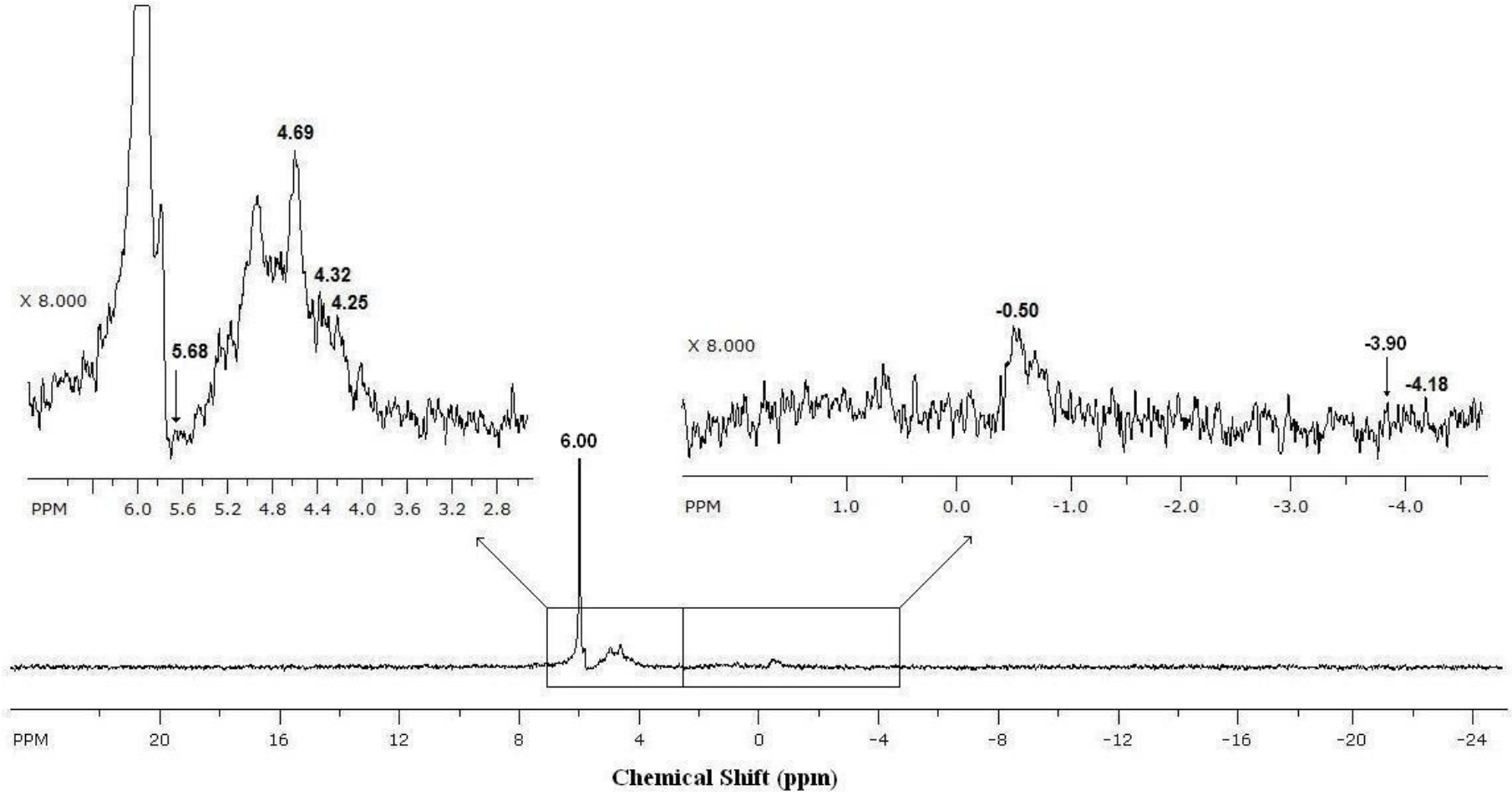

Figure 4.7. ^{31}P NMR spectra of Pond #11 extracted with NaOH–EDTA. Zoomed in (7ppm to 2.5ppm) and (2.5ppm to -4.5ppm)

4.3.2. Solution ^{31}P NMR Spectra Integration Analysis

After using the processing software SpinWorks 3.1.8 integrated the solution ^{31}P NMR spectra of the lake sediments samples (Cade-Menun 2005), the different phosphorus compounds in percentage composition of the total phosphorus (TP) in the lake sediments, obtained by total digestion method by Cade-Menun and Lavkulich (1997), are presented in Table 4.4. Solution ^{31}P NMR identifies inorganic P as orthophosphate in Blackstrap #3, Blackstrap #6, Pond #3 and Pond #11, which were accounted for 34.3%, 66.4%, 36.8% and 31.9% of the TP, respectively.

The sediments profiles from Blackstrap and Pond suggested that phytic acid accounted for less 6% of the TP. Phytic acid seems to be resistant towards degradation in NaOH solutions (Reitzel et al. 2009). Also, phytic acid is a common compound in freshwater sediment (Jørgensen et al. 2011). Release to the overlying water of phytic acid in a dissolved form may stimulate lake water production if P can be liberated from the compounds. Furthermore, with a concomitant release of phosphate to the lake water, a direct mineralization of the components can occur in the sediments.

The percentage of DNA in Blackstrap #3, Blackstrap #6, Pond #3 and Pond #11 were small, which were 1.2 %, 0.7 %, 3.2% and 3.9 % of TP, respectively. DNA might reflect microbial activity and supply of autochthonous matter (such as for example phytoplankton) (Reitzel et al. 2006). The higher amounts of DNA in the sediments from Pond could then suggest more active sediments with a higher abundance of bacteria or a higher input from autochthonous material as compared with the sediments from Blackstrap. Along with DNA in the sediments from Pond and Blackstrap, pyrophosphate was also found. Presence of pyrophosphates and some orthophosphate may also be due to breakdown products of polyphosphates in the NaOH extract (Hupfer et al. 1995).

Table 4.4. Percentage composition of total phosphorus in solution ^{31}P NMR spectroscopy

Sediment	Ortho-P%	Mono-esters%	Di-esters%	Pyro-P%	Polyp-end%	Other%
Blackstrap#3	34.3	24.8	10.0	0.7	0	30.2
Blackstrap#6	66.4	13.0	3.6	0.3	0	16.7
Pond#3	36.8	24.1	14.5	0.4	1.4	22.8
Pond#11	31.9	28.3	14.0	0.5	0.5	24.8

Polyphosphates can be synthesized by algae, bacteria, and fungi, probably as a response to changing redox conditions (Davelaar 1993). The low amount of polyphosphates in the sediment from Pond probably reflects a low supply of labile organic matter from the water column and hence a low microbial activity.

4.3.3. Discussion of the Results of Solution ^{31}P NMR and Chemical Sequential Extraction

The Jensen and Thamdrup (1993) method estimates organic P in lake sediments as the difference between TP and soluble reactive P in NaOH fraction. However, it does not provide information about specific chemical forms of organic P in lake sediments. In Table 4.5, the relative percentage of organic P in Blackstrap #3 detected by chemical sequential extraction was 20.3%. Whereas the sum of relative percentage of orthophosphate diesters and orthophosphate monoesters obtained by solution ^{31}P NMR was 34.8%. Orthophosphate diesters and orthophosphate monoesters are categorized as organic P (Turner et al. 2005). Since pyrophosphate and polyphosphate are not included, it indicates that this is the most conservative estimation of organic P by solution ^{31}P NMR. Similarly, the percentage of organic P in Pond #11 found by chemical sequential extraction was 31.6 %; however, the sum of relative percentage of orthophosphate diesters and orthophosphate monoesters was 42.3% by solution ^{31}P NMR. These results suggest that the solution ^{31}P NMR detects more organic P compounds than chemical sequential method does.

Table 4.5. The relative percentages of organic P obtained by chemical sequential extraction and solution ^{31}P NMR spectroscopy

Sediment	Organic P % of CSE TP	Sum of Mono-and Di-esters P % of Total Digestion TP
Blackstrap#3	20.3	34.8
Blackstrap#6	9.1	16.6
Pond#3	26.2	38.6
Pond#11	31.6	42.3

Golterman (1982) also argued that strongly acid and alkaline were unsuitable for extraction because of the likelihood of hydrolytic breakdown. This is the reason why the chemical sequential extraction may underestimate organic P. Therefore, the estimation of organic P in lake sediments based on chemical sequential extraction by Jensen and Thamdrup (1993) may not hold true for all lake sediments, and such results should be interpreted with caution.

In addition, Kizewski et al. (2011) pointed out several concerns about chemical extractions: (i) extraction can alter the P species chemically, such as the degradation of phosphate minerals; (ii) organic P species cannot be identified correctly because of changes in chemical shifts of P species in extraction solutions; and (iii) the distribution of species in extracts can be different from the distribution in the original sample due to selective extraction of certain species. In summary, the chemical sequential extraction proposed by Jensen and Thamdrup (1993) may have some shortcomings in representing the organic phosphorus fractionation in sediments.

4.4. Phosphorus (P) K-edge XANES Data Processing

P K-edge XANES was conducted on freeze-dried sediments samples, some of the chemical sequential extractions supernatants and some of the residues after chemical sequential extraction. Since P concentrations were low in the supernatants and the residues of NaCl, BD and residue fractions, no XANES spectra signals were obtained. Table 4.6 shows a summary of what samples had been studied by P K-edge XANES, how samples were treated and the figure numbers of the results. The discussion of the results will be in the next follow sections.

Table 4.6. Summary of treatment and results for samples studied by P K-edge XANES spectroscopy

Experiment/Analysis	Samples	Treatment for samples	Figure #
P K-edge XANES spectra of sediments samples	Sediments samples of Blackstrap #3, Blackstrap #6, Pond #3, and Pond #11	1 gram of well-mixed wet sediments samples were freeze-dried, and ground to powder	4.8, 4.9
Fingerprinting analysis of sediments samples	Sediments samples of Blackstrap #3, Blackstrap #6, and Pond #11	1 gram of well-mixed wet sediments samples were freeze-dried, and ground to powder	4.10, 4.12, 4.11
Fingerprinting analysis of residue after HCl extraction	Residue after HCl extraction of Blackstrap #6	1 gram of well-mixed wet sediments sample were conducted chemical sequential extraction (CSE) first, then the residue after HCl extraction was collected, freeze-dried and ground to powder	4.13
P K-edge spectra of supernatants in different fractions of sediments samples	Supernatants of NaOH fraction and HCl fraction of Blackstrap #3; supernatants of NaOH fraction and HCl fraction of Pond #11	15 gram of well-mixed wet sediment samples were conducted CSE first, the supernatants of NaOH and HCl fractions were collected, freeze-dried and ground to powder	4.14, 4.15, 4.18, 4.19
Fingerprinting analysis of supernatants in different fractions of sediments samples	Supernatants of NaOH fraction and HCl fraction of Blackstrap #3; supernatants of NaOH fraction and HCl fraction of Pond #11	15 gram of well-mixed wet sediment samples were conducted CSE first, the supernatants of NaOH and HCl fractions were collected, freeze-dried and ground to powder	4.16, 4.17, 4.20, 4.21

4.4.1. P K-edge XANES Spectra of Different Sediments Samples

The XANES spectra of freeze-dried sediments are shown in Figure 4.8. These spectra were background subtracted but not normalized. It is observed that the spectra are very noisy because of the low P concentrations in the sediments. Kelly et al. (2008) also suggested that spectral noise increases with decreasing total P concentration for any system.

In Figure 4.8, P K-edge XANES spectra of Blackstrap #3 and Blackstrap #6, Pond #3 and Pond #11 all consist of a white-line peak at dashed line (a). This peak occurs at approximately 2154.0 eV on each spectrum. Peak (a) was generated by a dipole-allowed electron transition from the 1s core level to the 3p antibonding orbital (Singh and Gräfe 2010). Brandes et al. (2007) suggested the XANES spectra of all phosphorus minerals have principal K edge peak energies between 2152.8 to 2154.1 eV. Thus, these white line peaks in the spectra stands for P K-edge peaks. Also, the data is very noisy from 2157.5 eV to the end of the post edge region of the spectra. No peaks can be discerned in the post edge region because the total concentration of P is too low and there are no corresponding peaks between the four spectra.

The spectra of Figure 4.9 were background subtracted and were normalized through the post-edge region 2180 to 2190eV of the averaged XANES files. Thus, the relative peak heights and intensity of the spectra correspond to the amount of P in the sediments samples (Beauchemin et al. 2003). The intensities of the spectra of Pond #3 and Pond #11 are higher than those of Blackstrap #3 and Blackstrap #6. It suggests the amount of P in Blackstrap sediments is lower than those in Pond sediments, which is consistent with the result of the chemical sequential extraction in Table 4.2. Pond #3 has the largest amount of P, probably because P is heterogeneously distributed within a sample.

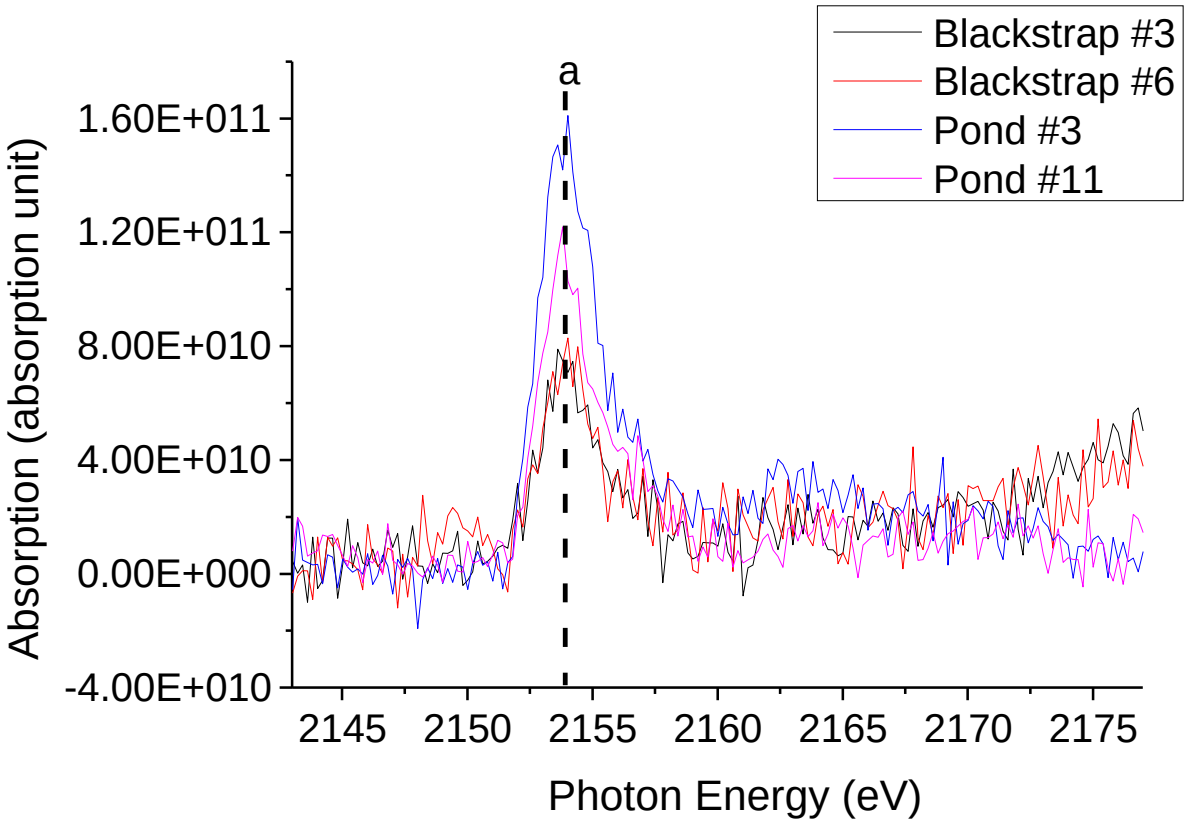

Figure 4.8. Background subtracted P K-edge XANES spectra of freeze-dried sediments samples powder (Pink: Pond #11, blue: Pond #3, red: Blackstrap #6, and black: Blackstrap #3)

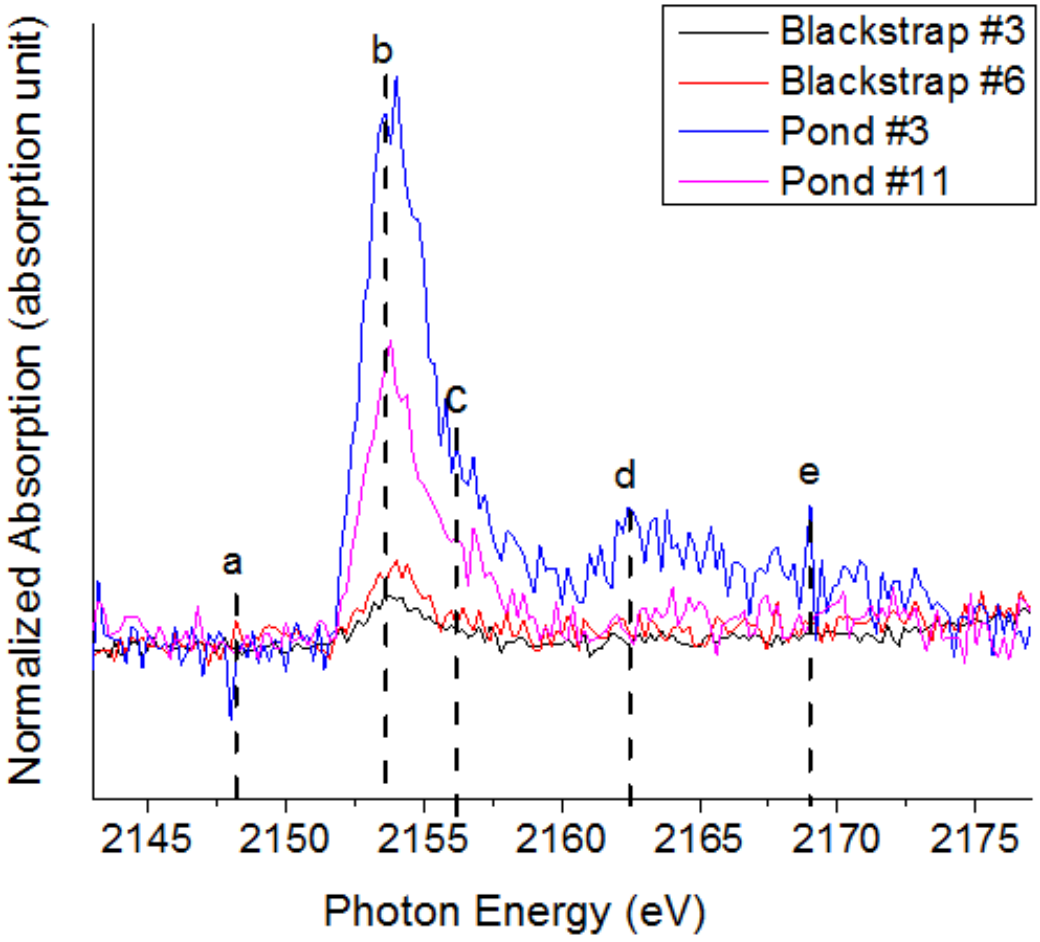

Figure 4.9. Normalized P K-edge XANES spectra of freeze-dried sediments samples powder (Pink: Pond #11, blue: Pond #3, red: Blackstrap #6, and black: Blackstrap #3)

4.4.2. P K-edge XANES Fingerprinting Analysis of Different Sediments Samples

The 'fingerprinting' approach analysis by comparing the features of sample spectrum to the features of reference spectrum was used for identifying the P species in the sediments. For the sediments sample of Blackstrap #3, a post-edge shoulder (b) at approximately 2156 eV and a subtle post-edge (c) feature around 2163 eV were found in the spectrum, which to some extent agreed with spectral features of apatite (Figure 4.10). The spectral features of Ca phosphates are confirmed by Hesterberg et al. (1999), Sato et al. (2005), and Ingall et al. (2010). In Figure 4.10, the shape of spectrum of Blackstrap #3 is similar to the shape of spectrum of phytic acid. Thus, it may indicate the presence of phytic acid in the sediments sample of Blackstrap #3.

For Pond #11, a post-edge shoulder (b) at approximately 2156 eV and a subtle post-edge (c) feature around 2163 eV, and an oxygen oscillation peak (d) at 2170 eV were found in the spectrum, which suggests the existence of apatite (Figure 4.11). However, this spectrum may also indicate the presence of phytic acid and $AlPO_4$. It's reasonable to suggest the presence of phytic acid in the spectrum, because the solution ^{31}P NMR spectroscopy in this study has already pointed out that all of the four sediments samples contained phytic acid.

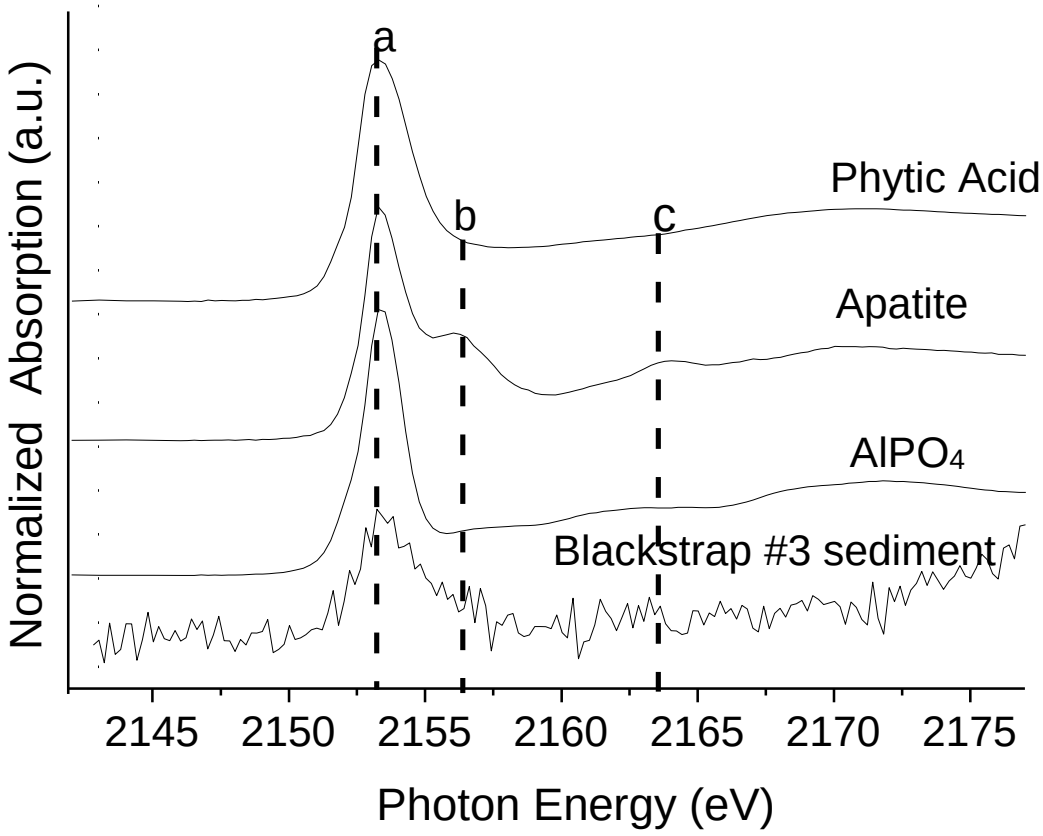

Figure 4.10. Normalized P K-edge XANES spectra of Blackstrap #3 fingerprinting with Phytic Acid, $AlPO_4$ and Apatite

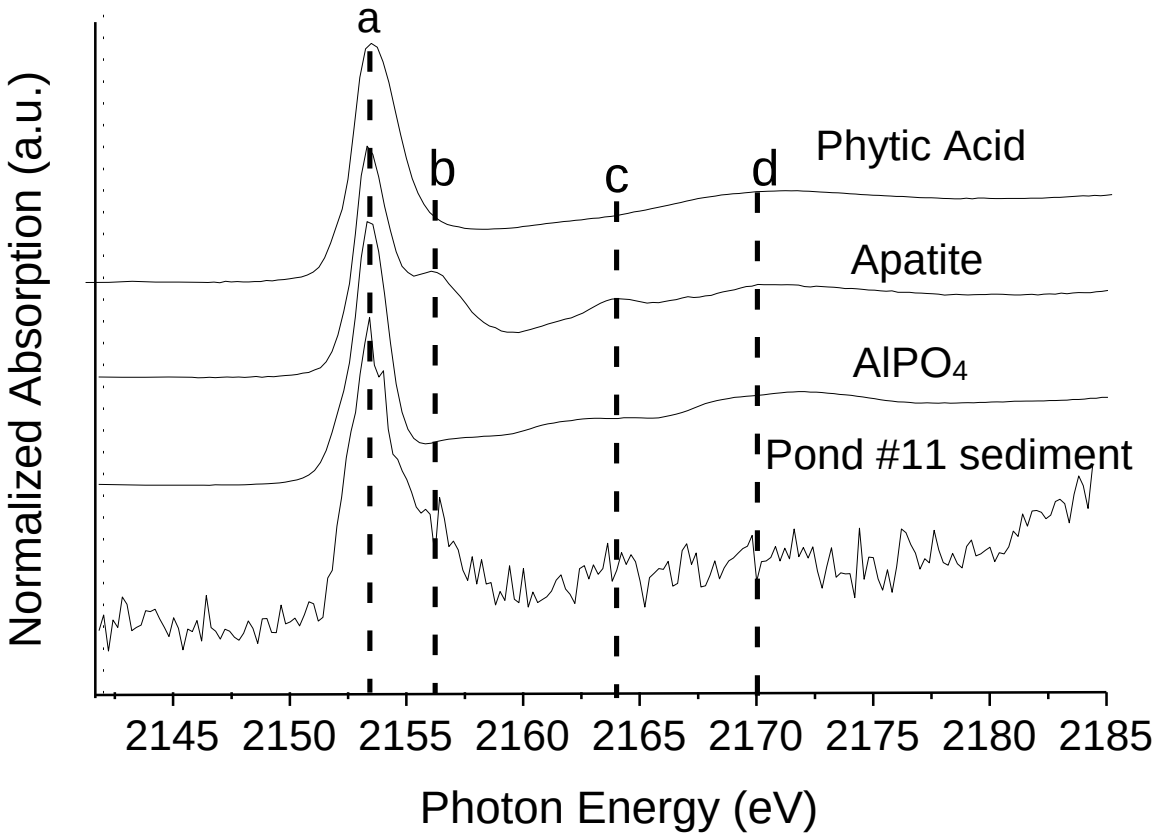

Figure 4.11. Normalized P K-edge XANES spectra of Pond #11 fingerprinting with $AlPO_4$, Apatite, and Phytic Acid

4.4.3. P K-edge XANES Fingerprinting Analysis of HCl Residue in Sediments Sample

Since the spectra of sediments were very noise, not all residue of each fraction in the sediment samples were analyzed by P K-edge XANES. The detection limit of P K-edge XANES is 100 hundred ppm (Li et al. 2014). Ca-P was accounted for the large proportion in the sediments samples according to the result of the chemical sequential extraction. Therefore, only the residue after HCl extraction of Blackstrap #6 was freeze-dried and was analyzed by P K-edge XANES.

For the sample of Blackstrap #6 sediments (Figure 4.12), a post-edge shoulder (b) at approximately 2156 eV and a subtle post-edge (c) feature around 2163 eV, and a peak (d) around 2170 eV were shown in the spectrum, indicating the presence of apatite. But the spectrum of Blackstrap #6 residue after HCl extraction (Figure 4.13) to some degree matched with the spectral feature of apatite as well. This result indicates HCl may not be able to extract apatite. The reason is that apatite is the most thermodynamically stable form of CaP and is less soluble (Ajiboye 2007). In other words, the chemical extraction method proposed by Jensen and Thamdrup (1993) may underestimate Ca-P in the lake sediments.

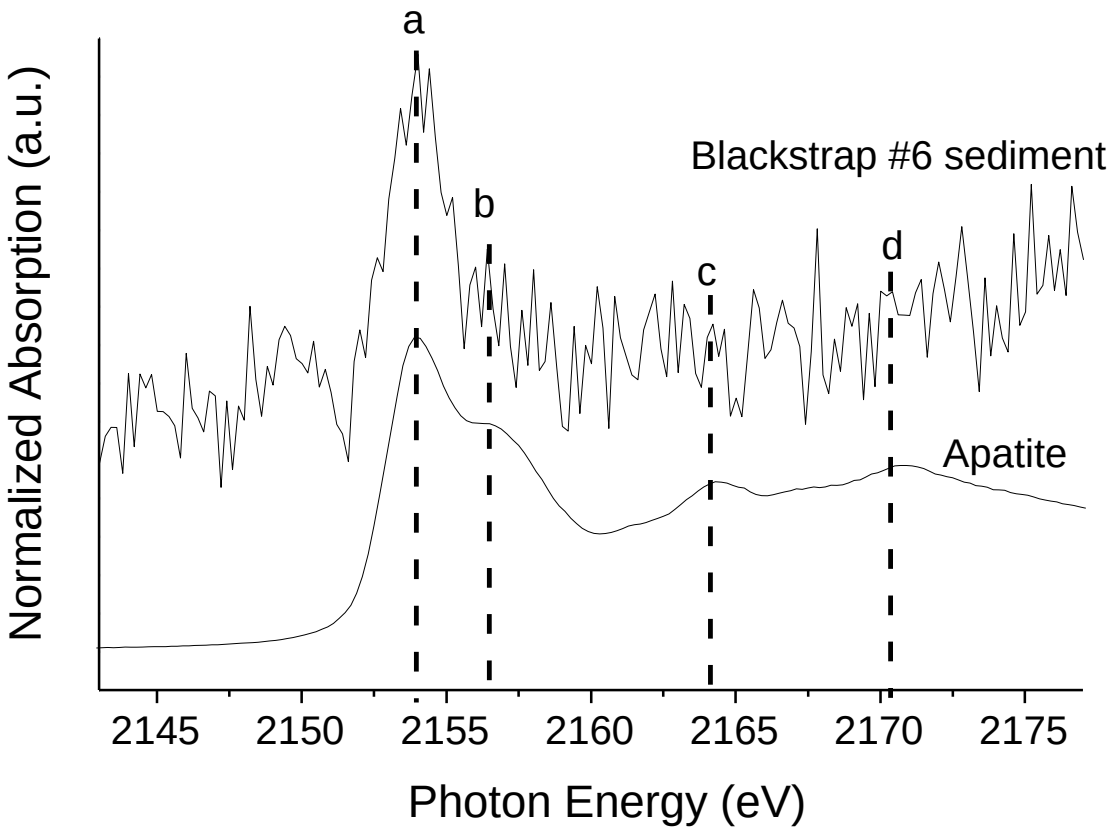

Figure 4.12. Normalized P K-edge XANES spectra of Blackstrap #6 sediment fingerprinting with Apatite

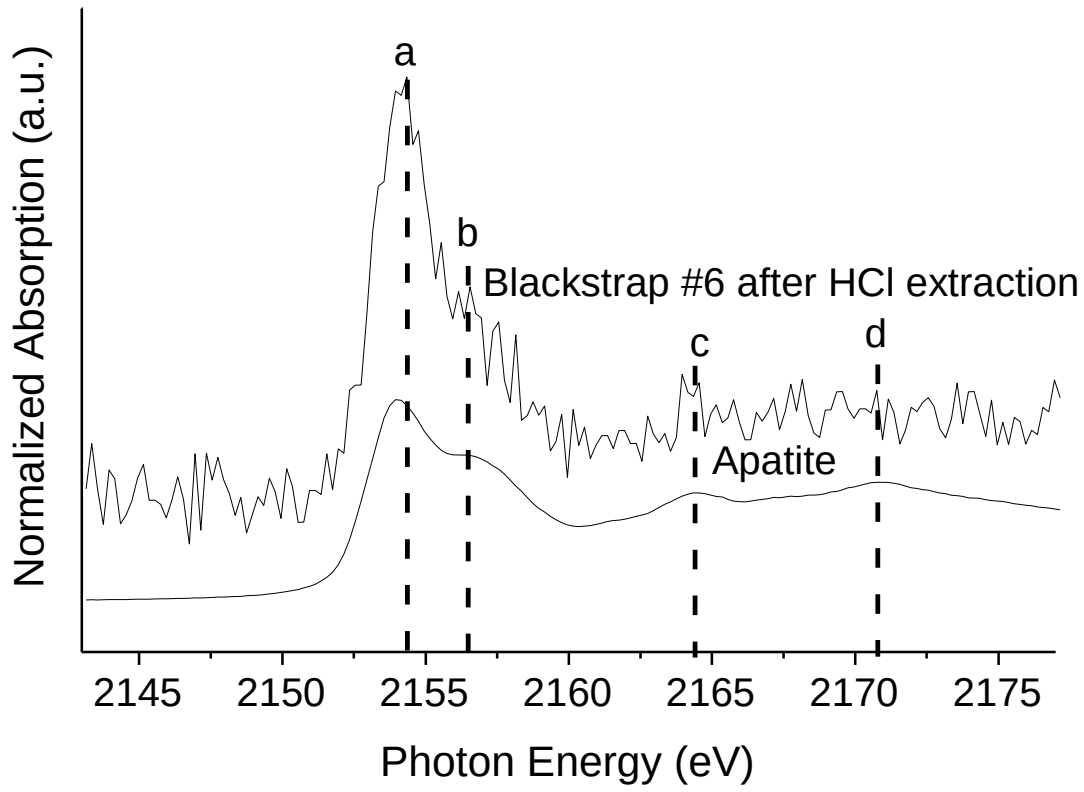

Figure 4.13. Normalized P K-edge XANES spectra of 1 g Blackstrap #6 residue after HCl extraction fingerprinting with Apatite

4.4.4. P K-edge XANES Fingerprinting Analysis of Supernatants in Different Fractions of Sediments Samples

The results of P K-edge XANES fingerprinting analyses of freeze-dried sediments and the residue after HCl extraction could be uncertain to some degree, since there was considerable noise in the spectra. In order to enhance the quality of spectra as well as the reliability of results, it is necessary to increase the P concentration in the supernatant of each chemical extraction fraction. To achieve this without changing the amount of extractant, 15 grams instead of 1 gram of well-mixed wet sediments samples were used in the chemical sequential extraction, and then freeze-dried for conducting XANES analysis.

The XANES spectra of freeze-dried supernatants of NaOH and HCl fraction of Blackstrap #3 are shown in Figure 4.14 and Figure 4.15. In Figure 4.14, the white line peak (a) is situated at 2152.4 eV in the spectrum of the supernatant of NaOH fraction, while the white line peak (b) is located at 2152.8 eV in the spectrum of the supernatant of HCl fraction. The reason why the energy positions of the white line peaks are different is due to the charge, electronegativity, and interatomic distance of the coordinating action in different P compounds (Franke and Hormes 1995). Different phosphorus compounds were shown in the NaOH and HCl fraction. Based on the Jensen and Thamdrup (1993) method, Al-P may be presented in the supernatant of NaOH fraction, and Ca-P may be shown in the supernatant of HCl fraction. Different compounds have different structural and chemical environment. This made the photon energy shifts between the spectra of NaOH and HCl fractions.

The white line peak positions of spectrum of NaOH and HCl fractions are different from the white line peak positions of phosphate minerals reported by Ingall et al. (2010). The peak energies of apatite minerals are 2153.0 ± 0.1 eV, and the peak energies of aluminum phosphate minerals are 2153.6 ± 0.2 eV (Ingall et al. 2010). It is possible that the X-ray microfocus beamline (2-ID-B at the Advanced Photon Source) used in the research by Ingall et al. is different from the Soft X-ray Microcharacterization Beamline (SXRMB) (06B1-1 at the Canadian Light Source's) used in this study. Thus, it cannot identify the specific forms of P in the supernatants of NaOH fraction or HCl fraction based on the white line peak positions of the spectrum. The fingerprinting analysis, which may provide more information about the chemical forms of P, will be discussed later.

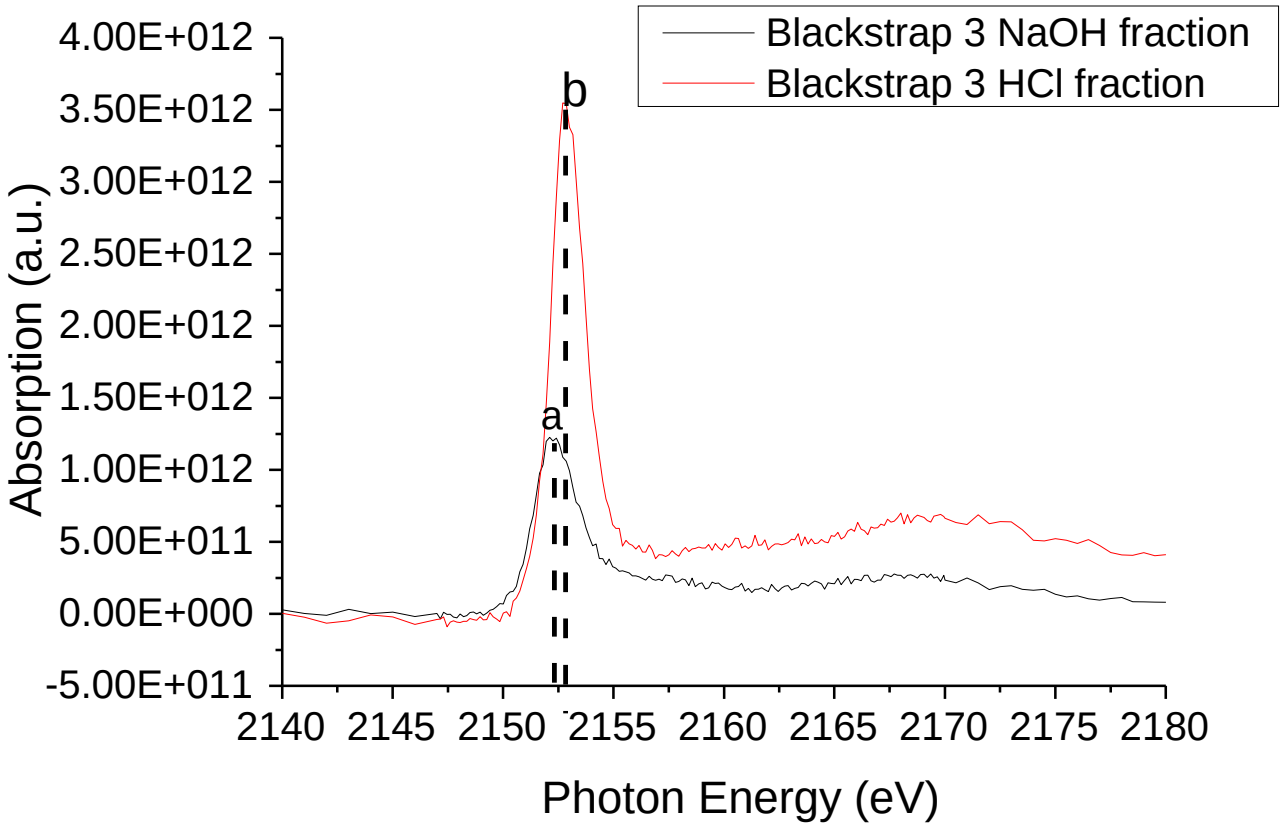

Figure 4.14. Background subtracted P K-edge XANES spectra of freeze-dried 15g Blackstrap #3 supernatants (Black: NaOH fraction; Red: HCl fraction)

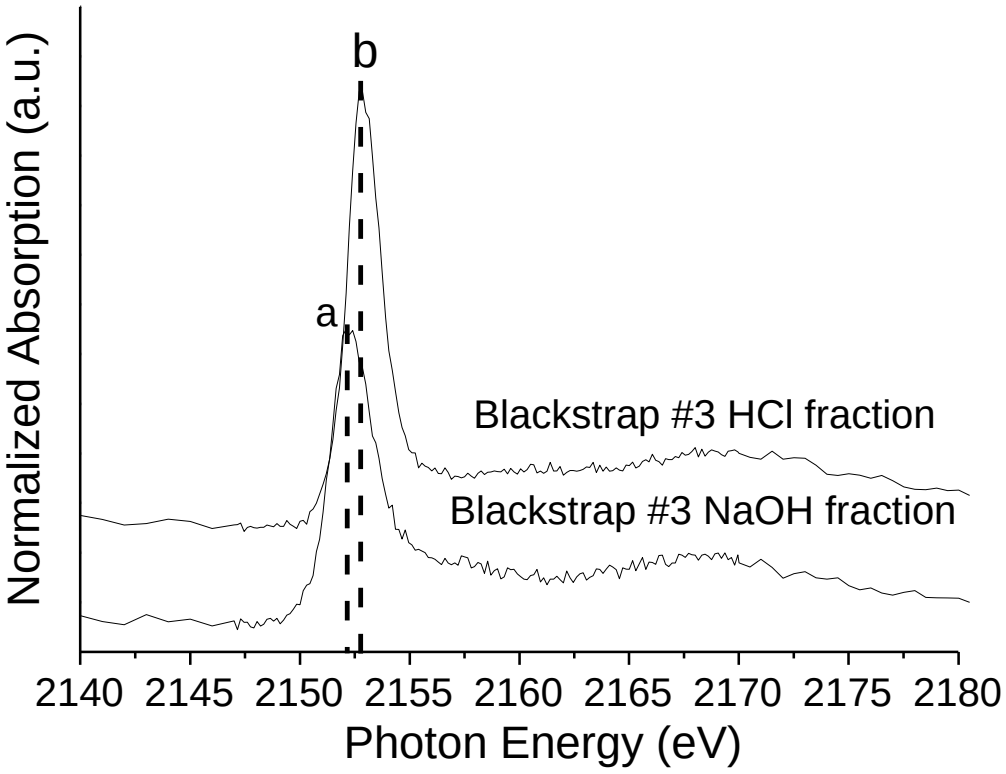

Figure 4.15. Normalized P K-edge XANES spectra of freeze-dried 15g Blackstrap #3 supernatants

In Figure 4.15, the relative peak height of the spectrum of HCl fraction is higher than that of the spectrum of NaOH fraction. Since the spectra were normalized through the post-edge region 2180 to 2190 eV, the relative peak heights and intensity of the spectra correspond to the amount of P in the supernatants (Beauchemin et al. 2003). Therefore, P concentration in the supernatant of HCl fraction is larger than that in the supernatant of NaOH fraction. It is consist with the results of chemical sequential extraction in Table 4.2.

A broadening of the white line peak (2152.4 eV) and a wide oxygen oscillation peak (d) is around 2170 eV in the spectrum of NaOH fraction (Figure 4.16), which indicates the presence of phytic acid. These spectral features of phytic acid can also be observed by Peak (2002). Other studies show the organic P compound's white line peak is around 2152-2152.25 eV (Kruse and Leinweber 2008) and have a broad absorption peak between 2160 eV and 2170 eV (Brandes et al. 2007). Also, according to the previous solution ^{31}P NMR results in this study, the sediment samples contained phytic acid. Thus, it's reasonable to suggest there is phytic acid in the supernatant of NaOH fraction, which means NaOH can extract phytic acid from the sediments. Besides, the solution ^{31}P NMR spectroscopy detected DNA in sediments samples. However, it's difficult to identify different organic P by using XANES analysis, because organic forms of phosphate have very similar spectra (Peak et al. 2002; Beauchemin et al. 2003).

In Figure 4.16, neither the subtle spectral feature of $AlPO_4$ reference at peak (b) 2156 eV nor peak (c) 2162 eV were found in the spectrum of NaOH fraction. It indicates that no $AlPO_4$ in the supernatant of NaOH fraction. Nevertheless, the spectra of various reference compounds, such as aqueous phosphate (Sato et al. 2005), phosphate adsorbed on aluminum oxides such as adsorbed P on amorphous $Al(OH)_3$ and gibbsite (Peak et al. 2002), and organic orthophosphate monoesters like phytic acid (Ajiboye 2007) were all similar, which lack of the features of phosphate found in the spectrum of $AlPO_4$. This means distinguishing between the phosphates adsorbed on aluminum oxides and phytic acid is very difficult. Also, the result of chemical sequential extraction indicated there was 53.13 mg/kg Al-P in Blackstrap #3. The Al-P concentration was below 100 ppm which is under detection limit of XANES (Li et al. 2014), which may result in no features of aluminum phosphates presented in the spectra. Thus, it makes conclusive aluminum phosphate speciation difficult regarding the XANES spectra of freeze-dried supernatants of NaOH fraction of Blackstrap #3.

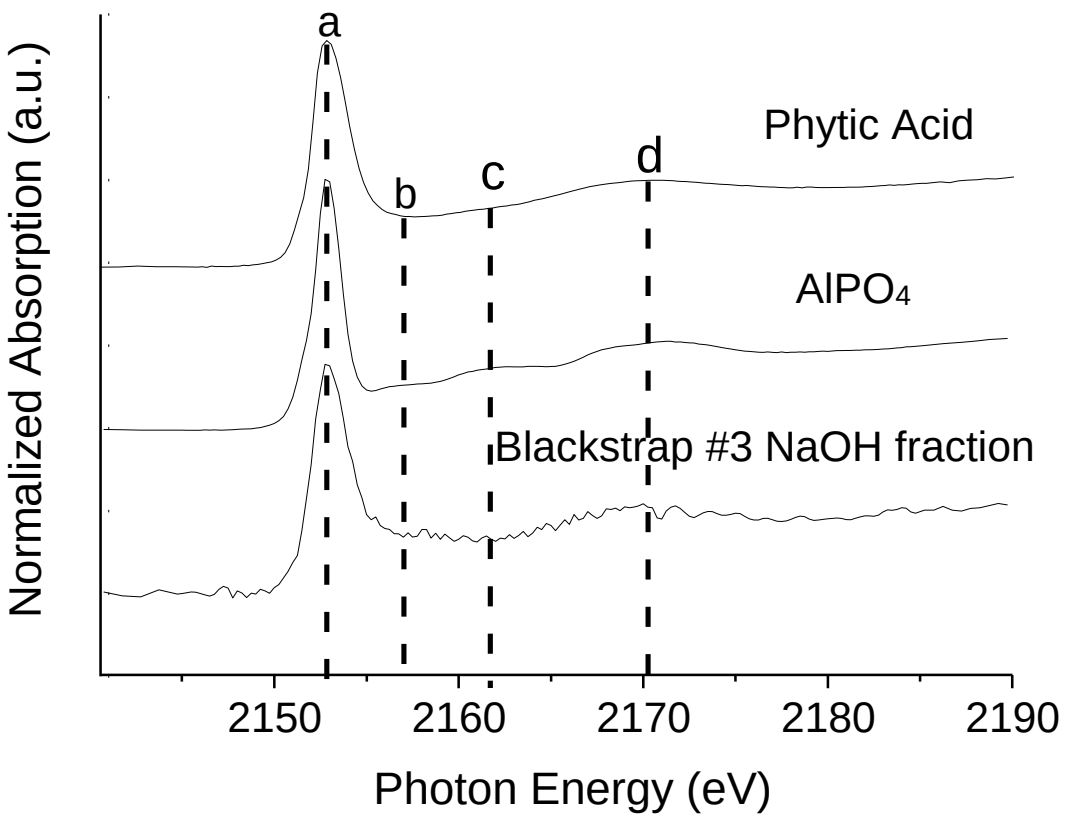

Figure 4.16. Normalized P K-edge XANES spectra of Blackstrap #3 NaOH fraction fingerprinting with $AlPO_4$ and Phytic Acid

In the spectrum of HCl fraction of Blackstrap #3 (Figure 4.17), neither the shoulder (b) at 2156 eV nor the post edge peak (c) at 2163 eV were shown in the spectrum, which may indicate no apatite in the HCl fraction. Nevertheless, the result of fingerprinting analysis of spectrum shown in Figure 4.10 indicated the presence of apatite in the Blackstrap #3 sediments sample. Therefore, it is reasonable to conclude that HCl is not able to extract apatite. The reason is that apatite is the most thermodynamically stable form of CaP and is less soluble (Ajiboye 2007). In other word, the chemical sequential extraction proposed by Jensen and Thamdrup (1993) may underestimate Ca-P in the lake sediments. Although the spectrum of HCl fraction of Blackstrap #3 doesn't share the key features with apatite spectrum, it has some subtle spectral features at 2156 eV and 2162 eV, indicating this HCl fraction contains $AlPO_4$. The reason is that the volume of NaOH extract from the third step is not enough to extract all $AlPO_4$.

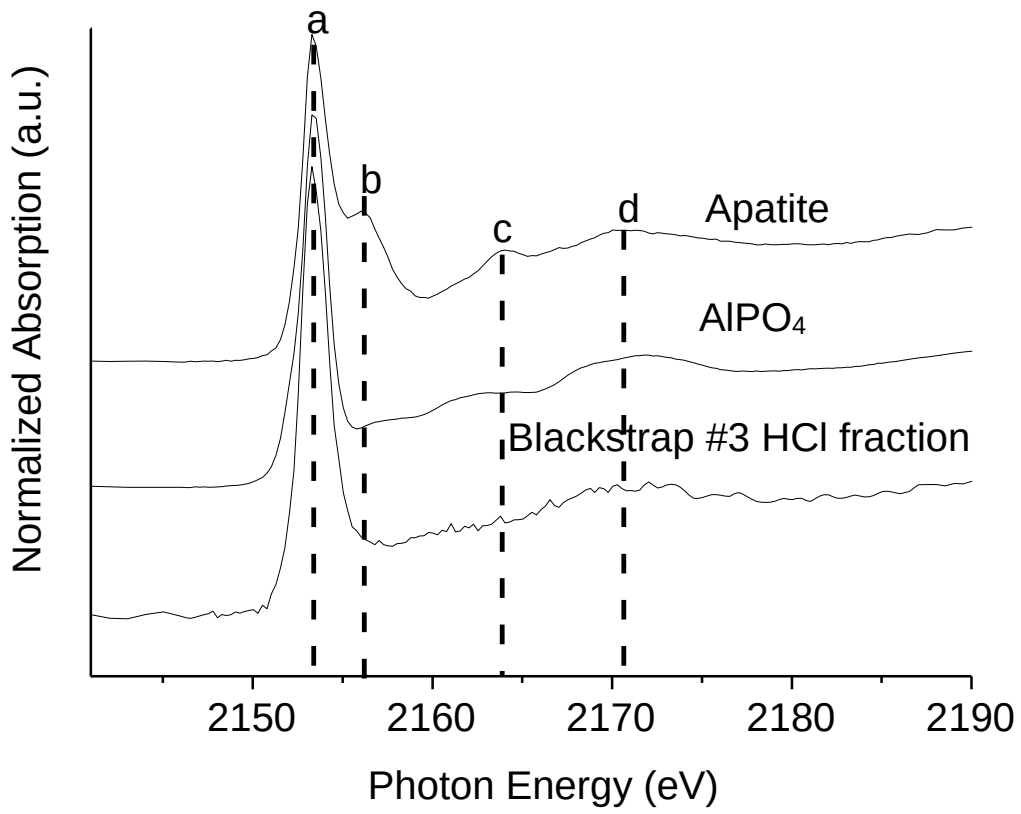

Figure 4.17. Normalized P K-edge XANES spectra of Blackstrap #3 HCl fraction fingerprinting with Apatite and $AlPO_4$

The XANES spectra of freeze-dried supernatants of NaOH and HCl fraction of Pond #11 are shown in Figure 4.18 and Figure 4.19. In Figure 4.18, similar to the spectra of freeze-dried supernatants of NaOH and HCl fraction of Blackstrap #3 (Figure 4.14), the white line peak (a) is situated at 2152.4 eV in the spectrum of the supernatant of NaOH fraction, while the white line peak (b) is located at 2152.8 eV in the spectrum of the supernatant of HCl fraction.

In Figure 4.19, the relative peak height of the spectrum of HCl fraction is larger than that of the spectrum of NaOH fraction. Since the spectra were normalized through the post-edge region 2180 to 2190 eV, the relative peak heights and intensity of the spectra correspond to the amount of P in the supernatants (Beauchemin et al. 2003). It indicates P concentration in the supernatant of HCl fraction is larger than that in the supernatant of NaOH fraction of Pond #11. It is consist with the results of chemical sequential extraction in Table 4.2.

For the NaOH fraction of Pond #11 (Figure 4.20), an oxygen oscillation peak (e) at 2170 eV was shown, but neither subtle feature at peak (c) 2156 eV nor peak (d) 2162 eV were found in the spectrum. It indicates no $AlPO_4$ is shown in the NaOH fraction of Pond #11. Nevertheless, the spectrum of NaOH fraction is some degree similar to the spectrum of phytic acid, which suggests the presence of phytic acid in the NaOH fraction of Pond #11. It is consistent with the result of the solution ^{31}P NMR which detected phytic acid in Pond #11.

Similar to the spectrum of HCl fraction of Blackstrap #3, neither the should (b) at 2156 eV nor the post edge peak (d) at 2163 eV were found in the spectrum of HCl fraction of Pond #11 (Figure 4.21), but an oxygen oscillation peak (e) at 2170 eV was shown in the spectrum. The spectrum may suggest no apatite presented in the HCl fraction of Pond #11. This result indicates that HCl may not extract apatite from the sediments samples. Different from the spectrum of HCl fraction of Blackstrap #3, no features of $AlPO_4$ were shown in the spectrum of HCl fraction of Pond #11.

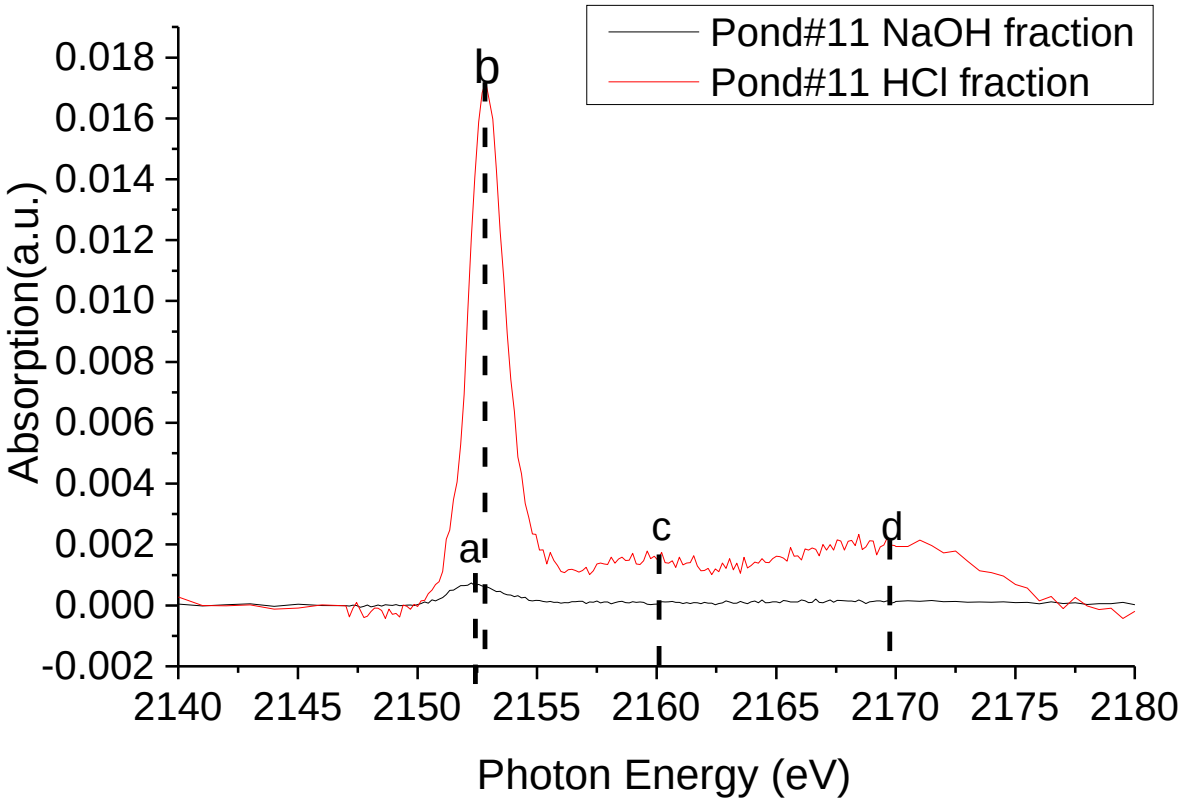

Figure 4.18. Background subtracted P K-edge XANES spectra of freeze-dried 15g Pond #11 supernatants (Black: NaOH fraction; red: HCl fraction)

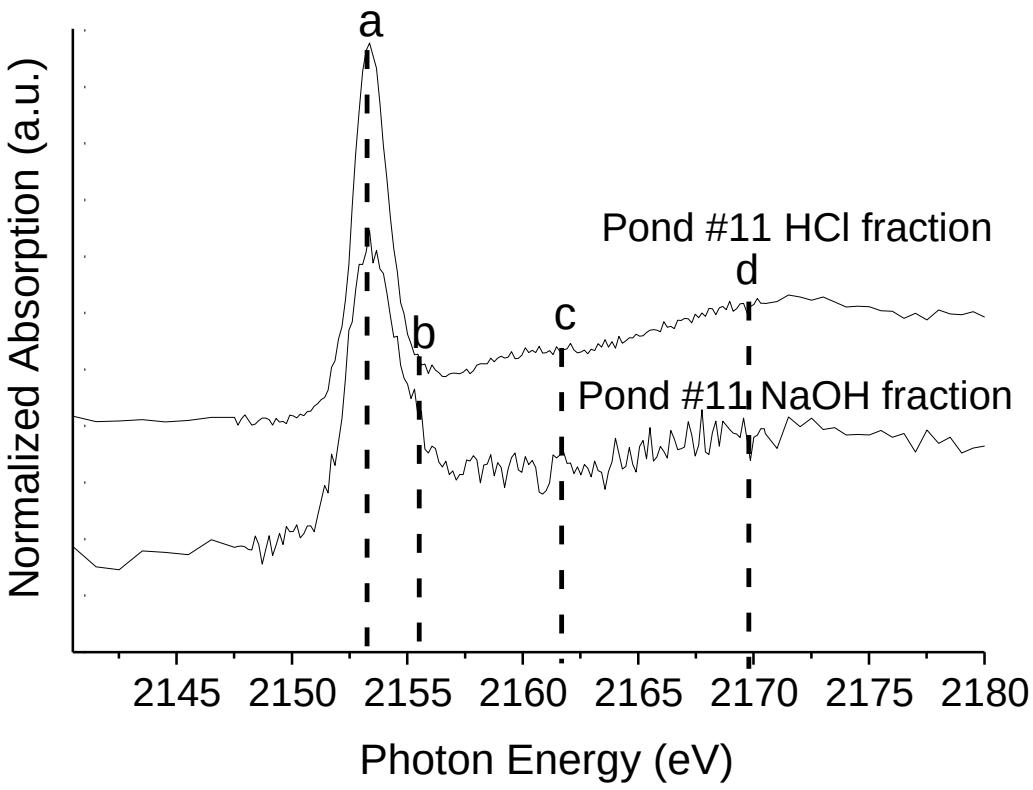

Figure 4.19. Normalized P K-edge XANES spectra of freeze-dried 15g Pond #11 supernatants

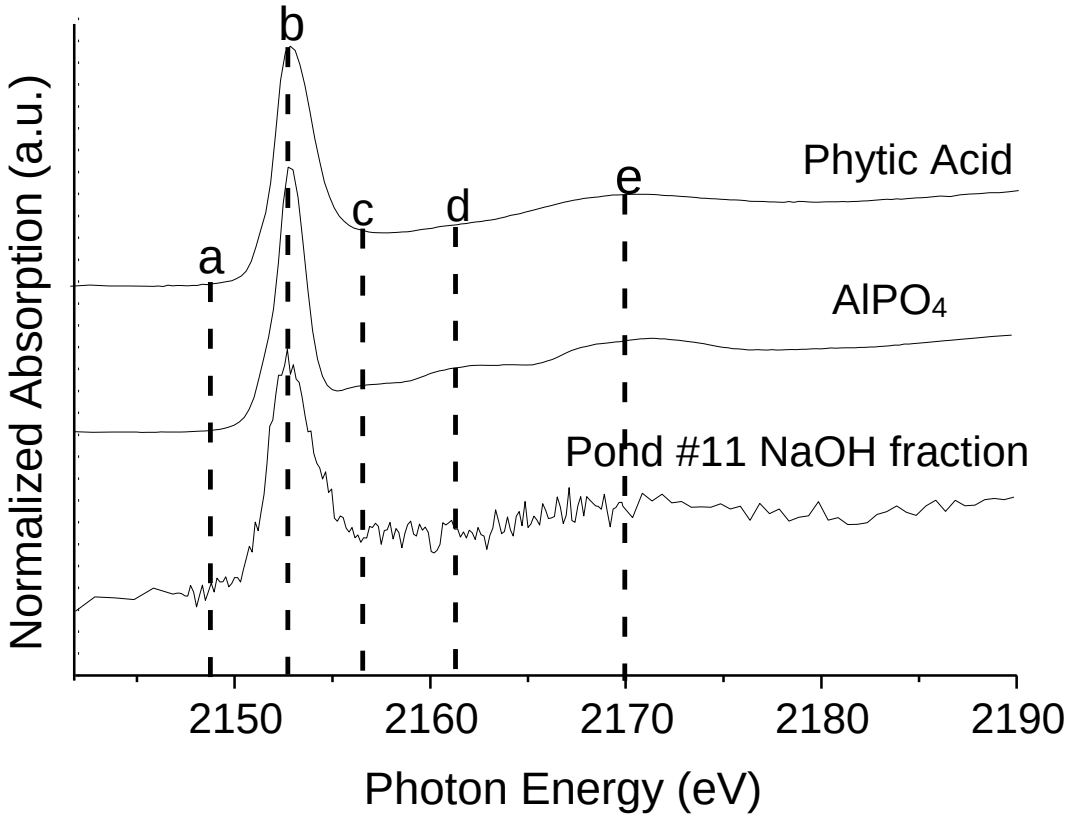

Figure 4.20. Normalized P K-edge XANES spectra of Pond #11 NaOH fraction fingerprinting with $AlPO_4$ and Phytic Acid

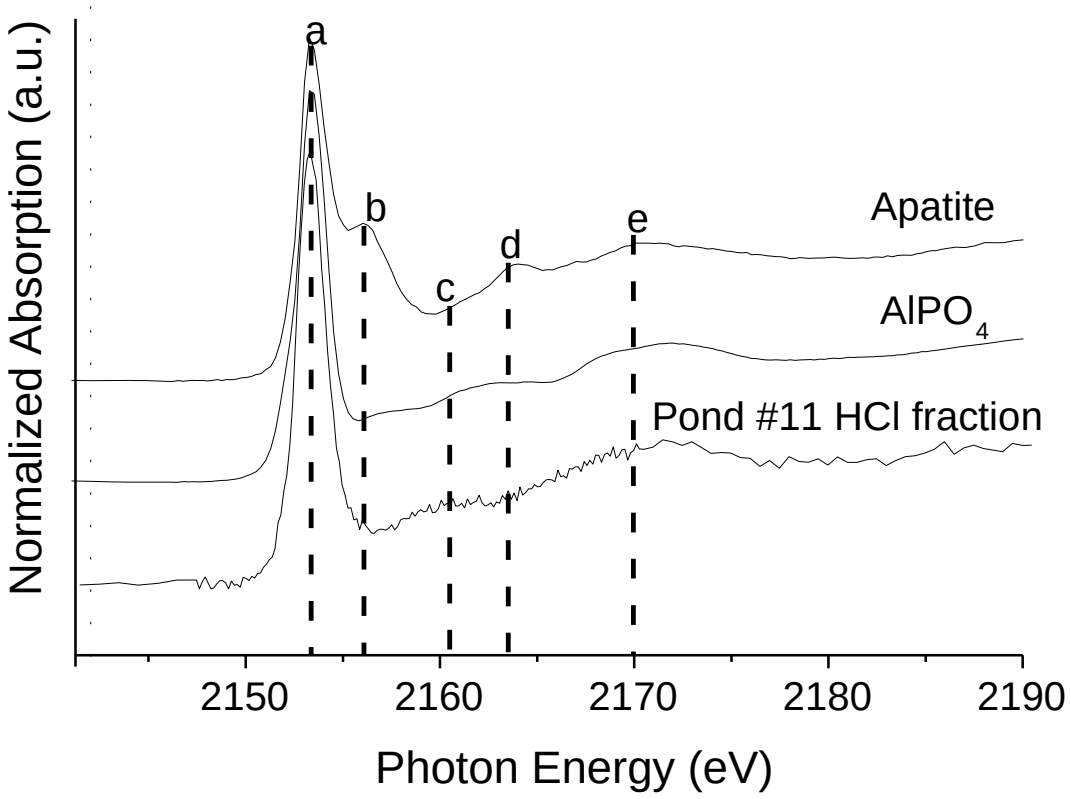

Figure 4.21. Normalized P K-edge XANES spectra of Pond #11 HCl fraction fingerprinting with Apatite and $AlPO_4$

4.5. Summary of Result and Discussion

To start with, the P fractionations of all of the sediments samples were studied using the chemical sequential extraction method. The chemical sequential extraction (CSE) can reveal the P fractionation in lake sediment even when the P concentration in the sediments sample is very low. Firstly, the chemical sequential extraction showed the amount of total P in Pond #3 and Pond #11 were larger than that in Blackstrap #3 and Blackstrap #6. Secondly, it indicated that inorganic P was dominant in all of the sediments samples. Thirdly, it suggested calcium-bound P accounted for the largest proportion of total P in every sediments sample.

All sediments samples were used to conduct experiments of the solution ^{31}P NMR with the NaOH-EDTA extracts. The solution ^{31}P NMR spectroscopy clearly identified orthophosphate, phytic acid, pyrophosphate, and polyphosphate in the sediments samples. In addition, the solution ^{31}P NMR spectroscopy detected more organic P than chemical sequential extraction. However, each fraction of the sediments sample was not studied by the solution ^{31}P NMR, because the P concentration was very low. Low P concentration usually results in low signal to noise ratio, which makes the assignment of peaks of P species difficult.

The P species in freeze-dried sediments samples were studied using the XANES. The result was that all of the sediments samples may contain apatite and phytic acid. The XANES only identified the phytic acid in all sediments, which was consistent with the result of the ^{31}P NMR analysis. However, the ^{31}P NMR also detected pyrophosphate, DNA and polyphosphate. Thus, it showed that the XANES has limitations in distinguishing between various organic P phases.

Although an attempt was made to identify the P species in each step of the sequential extraction procedure using XANES, it failed to obtain the spectra of the supernatants and residues of NaCl, BD and residue fractions due to the low concentrations of P in those fractions. However, the study still found apatite in the residue after the HCl extraction of Blackstrap #6. In addition, it indicated that no apatite in the supernatant of HCl fraction of both Blackstrap #3 and Pond #11. These results suggested that HCl might not be able to extract apatite.

CHAPTER 5 Conclusion and Recommendation

5.1. Conclusion

The major objective of this study was to enhance the understanding of the Jensen and Thamdrup (1993) chemical sequential extraction method for studying the sedimentary phosphorus fractionation by using solution ^{31}P NMR spectroscopy and phosphorus K-edge XANES spectroscopy.

The research using the chemical sequential extraction indicates that inorganic P is dominant in all sediments samples. Also, it suggests that calcium-bound P accounts for the largest proportion of the total P in every sediments sample. The solution ^{31}P NMR spectroscopy clearly identifies orthophosphate, phytic acid, pyrophosphate, and polyphosphate in the sediments samples. The phosphorus K-edge XANES spectroscopy shows all of the sediments samples contain apatite and phytic acid.

The study using the XANES identifies apatite in the residue after the HCl extraction of Blackstrap #6; however it indicates no apatite in the supernatant of HCl fraction of both Blackstrap #3 and Pond #11. Although an attempt was made to identify the P species in each step of the sequential extraction procedure using the XANES, it fails to obtain the spectra of the supernatants and residues of NaCl, BD and residue fractions due to the low concentrations of P in those fractions.

In general, this study illustrates a better understanding of the Jensen and Thamdrup (1993) chemical sequential extraction method for studying the sedimentary phosphorus fractionation by using solution ^{31}P NMR spectroscopy and phosphorus K-edge XANES spectroscopy. Chemical sequential extraction can reveal the P fractionation in lake sediment even when the P concentration in the sediments sample is very low. Unfortunately, it underestimates organic P as compared to the solution ^{31}P NMR spectroscopy. It also underestimates calcium-bound P when compared to the results of P K-edge XANES spectroscopy. Although the Jensen and Thamdrup

(1993) method has some shortcomings, it is still a good method for industrial practitioners to use. However, industrial practitioners should cautiously apply the Jensen and Thamdrup (1993) method to obtain P fractionation in lake sediments. In addition, the three techniques used in this study improve the understanding of P species in lake sediments. Spectroscopic techniques are a good addition to chemical sequential extraction techniques because they provide direct molecular and structural characterization of P speciation.

After studying sedimentary P fractionation, the next step is deciding on a method to restore eutrophic lakes. Possible methods include: sediments removal, deep water aeration (producing an oxidizing environment at the sediments surface), covering the sediments with an inert material or plastic sheeting, and recycling the sediments nutrients via bottom-feeding organisms.

Proper understanding of the sedimentary P fractionation may lead to an appropriate method for restoring eutrophic lakes by industrial practitioners. For example, the deep water aeration method is efficient for controlling P release from Fe-P rich sediments, since iron is present in ferric state precipitated in a colloidal structure when the sediment surface is oxidized, which prevents phosphate exchange. In this study, Ca-P accounts for the largest proportion of total P in every sediments sample. Thus, it is not suitable to use deep water aeration to control P release from the sediments from Blackstrap Lake or R.C.A.F pond.

In addition, organic P can be utilized by algae easily, so the underestimation of organic P leads to the ignorance of potential algae bloom. Underestimating Ca-P could result in inaccurate sedimentary P fractionation in lake sediments as well as misinterpretation of results after lake restoration. It is essential to have a better understanding of sedimentary P fractionation. Therefore, the industrial practitioners can choose the appropriate treatment to control P release from sediments and make a better decision after lake restoration.

5.2. Recommendation

A knowledge gap worth exploring is the underestimation of organic P likely due to the hydrolytic breakdown in the strong acid and alkaline extract. Investigating the kinetics of hydrolysis of specific organic P in strong acid and alkaline is recommended.

Another gap that needs to be explored is an appropriate method for enriching P concentrations in sediments but not changing the original P fractionation in the sediments. Low P concentration lake sediments samples make it difficult to obtain a high quality solution ^{31}P NMR or a high quality P-K edge XANES spectrum. To improve the extraction efficiency of P in lake sediments, the investigation of the best ratio of sediments to extractant is recommended.

The solution ^{31}P NMR spectra of each fraction of the lake sediments samples were not obtained in this study because of the low concentrations of P. It is recommended to enrich the P concentration in the supernatants before conducting the solution ^{31}P NMR. Also, it is recommended to use the residue of each fraction to perform the solution ^{31}P NMR with the NaOH-EDTA extracts. Therefore, it will provide a better understanding about the organic P phases in the supernatant and the residue in each step of the CSE.

While a direct comparison could not be made between the operationally defined P phases in all stages of the sequential extraction and P species identified by the XANES due to the concentration issue, further advances in optimizing the detection of low concentration species may make this possible in the nearer future. Based on the results of XANES analysis, it is recommended that

- A high quality of XANES spectrum resulted from higher P concentration in sediments. Therefore, it is recommended to use sediment with high P concentration or commercial reference material to conduct XANES analysis.
- A mixture of certified reference material, which is similar to the sample, should be investigated by XANES technique. This will make it easy for fingerprinting and liner combination fitting analysis.
- Proper sample preparation is helpful to acquire a good quality of XANES spectrum. Sediments sample should be mixed homogeneously, freeze-dried and grounded with a mortar and pestle before XANES analysis.
- It is recommended to use X-ray Fluorescence spectroscopy to understand the metal speciation in order to assign the inorganic P.

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APPENDIX

Table A. Water contents of sediments samples

Sediment sample	Tare weight(g)	(T+S) before(g)	(T+S) After(g)	Sample wet weight(g)	Sample dry weight(g)	Water loss(g)	Water contents (%)
Blackstrap#3	37.38	42.58	40.66	5.20	3.28	1.92	36.89
Blackstrap#6	33.61	41.53	38.77	7.92	5.16	2.75	34.78
Pond#3	36.68	40.43	37.59	3.75	0.91	2.84	75.73
Pond#11	39.58	41.78	40.27	2.20	0.68	1.51	68.88

Table B. Water contents of sediments samples duplicates results

Sediment sample	Tare weight(g)	(T+S) before(g)	(T+S) After(g)	Sample wet weight(g)	Sample dry weight(g)	Water loss(g)	Water contents (%)
Blackstrap#3	38.86	45.77	42.61	6.91	3.75	3.16	45.69
Blackstrap#6	35.83	40.82	39.09	4.99	3.26	1.73	34.61
Pond#3	37.85	40.45	38.71	2.60	0.86	1.74	66.95
Pond#11	37.08	41.20	38.44	4.12	1.36	2.76	66.98

Table C. Volatile solids in lake sediments samples

Sediment sample	Tare weight (g)	(T+S)before 60℃	(T+S)after 550℃	60℃ dry weight(g)	550℃ ash weight(g)	Volatile solids (g)	Volatile solids%
Blackstrap#3	37.38	40.66	40.56	3.28	3.18	0.10	3.15
Blackstrap#6	33.61	38.78	38.66	5.17	5.05	0.12	2.25
Pond#3	36.68	37.60	37.41	0.91	0.73	0.18	20.13
Pond#11	39.58	40.27	40.13	0.69	0.54	0.14	20.50

Table D. Volatile solids in lake sediments samples duplicates result

Sediment sample	Tare weight (g)	(T+S)before 60℃	(T+S)after 550℃	60℃ dry weight (g)	550℃ ash weight (g)	Volatile solids (g)	Volatile solids%
Blackstrap#3	38.86	42.62	42.41	3.76	3.55	0.21	5.50
Blackstrap#6	35.83	39.09	39.01	3.26	3.19	0.08	2.39
Pond#3	37.85	38.71	38.55	0.86	0.71	0.16	18.06
Pond#11	37.08	38.45	38.17	1.37	1.09	0.27	19.87

Table E. Phosphorus in NaCl fraction of lake sediments samples

Sediment	Sample volume (ml)	Sample dry weight(g)	NaCl SRP (mg/L)	NaCl SRP (mg/kg)	NaCl TP (mg/L)	NaCl TP (mg/kg)	NaCl NRP (mg/kg)
Blackstrap#3	50	0.71	0.05	3.71	0.09	6.33	2.62
Blackstrap#3	50	0.73	0.07	4.91	0.15	10.31	5.40
Blackstrap#6	50	0.70	0.02	1.43	0.08	5.86	4.43
Blackstrap#6	50	0.67	0.03	2.54	0.12	8.96	6.42
Pond#3	50	0.44	0.03	3.67	0.07	7.51	3.85
Pond#3	50	0.44	0.02	2.65	0.08	9.46	6.81
Pond#11	50	0.40	0.04	5.21	0.07	8.35	3.14
Pond#11	50	0.39	0.04	5.20	0.07	8.37	3.17

Table F. Phosphorus in BD fraction of lake sediments samples

Sediment	Sample volume(ml)	Sample dry weight(g)	BD SRP(mg/L)	BD SRP(mg/kg)	BD TP(mg/L)	BD TP(mg/kg)
Blackstrap#3	108	0.60	0.24	42.45	0.21	37.68
Blackstrap#3	108	0.63	0.28	47.69	0.28	47.31
Blackstrap#6	108	0.70	0.31	47.43	0.30	46.68
Blackstrap#6	108	0.68	0.30	47.28	0.31	48.30
Pond#3	108	0.29	0.29	106.32	0.24	88.15
Pond#3	108	0.29	0.31	114.03	0.27	99.38
Pond#11	108	0.33	0.30	96.75	0.22	73.18
Pond#11	108	0.33	0.28	91.72	0.27	87.60

Table G. Phosphorus in NaOH fraction of lake sediments samples

Sediment	Sample volume (ml)	Sample dry weight(g)	NaOH SRP (mg/L)	NaOH SRP (mg/kg)	NaCl TP (mg/L)	NaOH TP (mg/kg)	NaOH NRP (mg/kg)
Blackstrap#3	78	0.60	0.18	23.22	0.30	38.58	15.36
Blackstrap#3	78	0.60	0.18	23.05	0.87	113.96	90.91
Blackstrap#6	78	0.69	0.16	18.31	0.22	24.91	6.60
Blackstrap#6	78	0.68	0.14	15.96	0.22	25.73	9.76
Pond#3	78	0.30	0.20	53.76	0.44	116.44	62.68
Pond#3	78	0.30	0.17	44.00	0.65	170.20	126.20
Pond#11	78	0.33	0.19	45.34	0.47	109.72	64.39
Pond#11	78	0.34	0.15	35.17	0.94	216.25	181.08

Table H. Phosphorus in HCl fraction of lake sediments samples

Sediment	Sample volume (ml)	Sample dry weight (g)	HCl SRP (mg/L)	HCl SRP (mg/kg)	HCl TP(mg/L)	HCl TP (mg/kg)
Blackstrap#3	50	0.60	3.36	282.09	3.24	271.62
Blackstrap#3	50	0.60	3.14	262.54	2.94	246.09
Blackstrap#6	50	0.70	4.10	294.65	2.73	195.92
Blackstrap#6	50	0.71	4.46	314.95	3.57	252.24
Pond#3	50	0.30	1.27	214.37	1.37	231.93
Pond#3	50	0.33	1.47	220.17	1.45	215.87
Pond#11	50	0.32	1.31	202.34	1.18	182.05
Pond#11	50	0.34	1.34	198.80	1.30	193.20

Table I. Phosphorus in Residue fraction of lake sediments samples

Sediment	Sample volume (ml)	Sample dry weight (g)	Residue TP (mg/L)	Residue TP (mg/kg)
Blackstrap#3	100	0.59	0.21	36.53
Blackstrap#3	100	0.61	0.15	25.44
Blackstrap#6	100	0.71	0.18	25.39
Blackstrap#6	100	0.70	0.16	21.95
Pond#3	100	0.30	0.09	31.16
Pond#3	100	0.29	0.11	38.16
Pond#11	100	0.32	0.07	23.33
Pond#11	100	0.32	0.12	38.90

Table J. The sedimentary total phosphorus concentrations of sediments samples

Sediment	Dry weight(g)	TP dilute (mg/L)	TP(mg/L)	TP(mg/kg)
Blackstrap#3	1.50	0.55	2.76	460.75
Blackstrap#3	1.00	0.39	1.95	487.38
Blackstrap#6	1.50	0.61	3.03	504.25
Blackstrap#6	1.58	0.67	3.37	533.54
Pond#3	1.83	0.99	4.93	674.11
Pond#3	1.82	1.01	5.05	694.23
Pond#11	0.49	0.28	1.41	720.92
Pond#11	4.47	0.26	13.17	736.14

Table K. Different P concentrations shown in ^{31}P NMR solution state spectroscopy

Sediment	Total P	Ortho -P	Inorg -P	Mono -esters	Di -esters	Pyro -P	Polyp -end	Other	Org-P
	P concentration (mg/kg)								
Blackstrap #3	474.1	162.6	162.6	117.6	47.4	3.3	0.0	143.2	311.5
Blackstrap #6	518.9	344.5	344.5	67.5	18.7	1.6	0.0	86.7	174.3
Pond #3	684.2	251.8	251.8	164.9	99.2	2.7	9.6	156.0	432.4
Pond #11	728.5	232.4	232.4	206.2	102.0	3.6	3.6	180.7	496.1